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Exploring Racial Differences in Precocious Puberty among Girls: Implications for the Role of Environmental Factors

By

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Master of Public Health

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A.B.
Harvard University
2007

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2011

Abstract

Exploring Racial Differences in Precocious Puberty among Girls: Implications for the Role of Environmental Factors
By Dzifa Adjaye-Gbewonyo

A secular trend towards earlier pubertal onset has been documented in girls in the United States, and numerous studies have demonstrated racial differences in pubertal timing—specifically, earlier ages for pubertal landmarks in black girls compared to white girls—with indications that racial divergences may be a recent phenomenon. Neither of these observations has been fully explained, and few studies have examined clinically early puberty and racial differences in precocious puberty patients. This thesis therefore aimed to assess racial variations in the distribution of precocious puberty subtypes and in other patient characteristics through a review of medical records in order to better illuminate racial differences in puberty and possible pathways through which environmental exposures suspected to influence pubertal development may be contributing to observed patterns.

Results indicated substantial differences in the distribution of subtypes, though significant only at the 10% α level ($p=0.076$). Among 50 black patients, 26% had premature adrenarche, 28% had premature thelarche, 28% had central precocious puberty, and 18% had other forms of early puberty compared to 49%, 12%, 23%, and 16% in 49 white patients, respectively. A greater proportion of blacks were under 6 years than whites (42% vs. 12%), and blacks appeared younger on average in each subtype. The percentage of patients with potential neurologic causes of central precocious puberty was considerably greater in whites (46% vs. 14%). Racial differences in estradiol levels varied across subtypes. Additionally, low income and BMI were significantly higher in black patients overall and showed similar differences by subtype.

These results suggest that factors known to affect pubertal timing and to vary by race, such as BMI and income, do not account for racial differences in pubertal timing as they remain significantly different among only cases of early puberty. Moreover, the fact that most patients' puberty is not regulated by the true central mechanism, as in central precocious puberty, may support the notion that environmental influences are promoting hormonal activity outside of the main pathway. Moreover, a greater frequency of breast development and subtypes involving estrogen effects in black girls may support claims of higher estrogen-active environmental exposures among blacks.

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Acknowledgments

I would first like to thank my advisor Dr. Michele Marcus, whom I have worked for as a research assistant throughout my time at Rollins School of Public Health (RSPH). Working with her gave me the unique opportunity to take on this project and use it for my thesis. I would also like to thank Dr. Andrew Muir of Emory Children's Center (ECC) and the School of Medicine for collaborating on the project, which allowed us to use ECC records for the study, and for his expert advice on the data measures collected. Special thanks also go out to Dr. Jennifer Badik, ECC pediatric endocrinology fellow, for her involvement in the study and for helping me understand medical information from patient charts. I would further like to recognize Kathy Thornton, Natasha Quinones, and Sophelia Terry of ECC for supplying me with lists of patients and medical records.

I also want to extend my thanks to other members of the project team, including Drs. Matt Strickland and Lyndsey Darrow for laying the groundwork and for their role in the study design. Additionally, members of Dr. Marcus's research group deserve thanks for their suggestions during the research process, especially Dr. Mildred Maisonet and Metrecia Terrell who shared information from work on similar projects and provided SAS advice. I would further like to thank Dr. Owen Devine, a statistician at the Centers for Disease Control and Prevention (CDC) and adjunct faculty at RSPH, for his thesis data analysis course and the useful tools it provided, as well as Dr. Kevin Sullivan of RSPH and the CDC and Drs. Andrew Dean and Erik Knudsen from the CDC for guidance with Epi Info and with troubleshooting as I created the database for abstraction.

Lastly, I am forever grateful to my family, in particular my mother and sister, for their support not only throughout the thesis process but throughout my life in general.

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List of Abbreviations

AN	acanthosis nigricans
BCERC	Breast Cancer and Environmental Research Centers
BMI	body mass index
BPA	bisphenol A
CAH	congenital adrenal hyperplasia
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CNS	central nervous system
CP	cerebral palsy
CPP	central precocious puberty
DDT	dichlorodiphenyltrichloroethane
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone-sulfate
ECC	Emory Children's Center
FSH	follicle stimulating hormone
EDC	endocrine disrupting compound
GnRH	gonadotropin-releasing hormone
GnRHa	gonadotropin-releasing hormone agonist
HCHP	hormone-containing hair product
HIPAA	Health Insurance Portability and Accountability Act
HPG	hypothalamic-pituitary-gonadal

IRB	Institutional Review Board
IVF	in vitro fertilization
LH	luteinizing hormone
LOD	limit of detection
MAS	McCune Albright syndrome
MRI	magnetic resonance imaging
MRN	medical record number
NHANES	National Health and Nutrition Examination Survey
NHES	National Health Examination Survey
OPP	other precocious puberty
PA	premature adrenarche
PCOS	polycystic ovarian syndrome
PCP	personal care product
PPP	peripheral precocious puberty
PROS	Pediatric Research in Office Settings
PT	premature thelarche
PVL	periventricular leukomalacia
RR	risk ratio
SD	standard deviation
SES	socioeconomic status
TSH	thyroid-stimulating hormone
US	United States
WIC	Women Infants and Children

Chapter 1: Introduction

In the human life cycle, puberty is a significant time period of growth and change that marks the transition from childhood to adulthood. For girls, puberty typically begins with thelarche, the development of breasts, or pubarche, the growth of pubic hair (1). There is some variation in the pubertal sequence from person to person and across racial groups, with thelarche tending to occur prior to pubarche especially in white girls, while in black girls pubarche may occur earlier than or in closer proximity to thelarche (2). The onset of puberty is also accompanied by a linear growth spurt; and within an average of two years after puberty starts, menarche, or the first menstrual period, is attained, marking the beginning of fertility (3).

These physical changes are coordinated by a complex hormonal system referred to as the hypothalamic-pituitary-gonadal (HPG) axis, which is active prenatally and in early infancy and then remains inactive until it is again reactivated at the time of puberty (4). This is characterized by the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which then triggers the release of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary in a pulsatile fashion (1). These gonadotropins lead to the production of sex hormones by the gonads, in particular estradiol production by the ovaries in females. This is responsible for the bodily changes associated with puberty such as thelarche and menarche (1). The independent process of adrenarche also occurs around the time of puberty with the synthesis of androgens by the adrenal glands commencing several months to years prior

to the gonadotropic hormonal changes (5-6); and this drives the growth of pubic and axillary hair as well as other androgen-related changes such as body odor and acne (5).

1.1 Precocious Puberty

When the changes of puberty occur particularly early, the medical label of precocious puberty is given to describe it. Traditionally, this condition has been defined as the onset of puberty in girls before the age of 8 years or menarche before age 9 (7). The term encompasses a spectrum of conditions that can be divided into three major categories: incomplete precocious puberty—which is not dependent on gonadotropins and is considered a variation of normal puberty with only isolated signs developing early—central precocious puberty, which is gonadotropin-dependent, and peripheral or gonadotropin-independent precocious puberty (3).

Within the category of incomplete precocious puberty, premature thelarche refers to the isolated development of breast tissue without progressive puberty. It tends to be most commonly found in infants and toddlers, and can be associated with ovarian cysts or elevated estradiol levels (5, 8). In a minority of these cases, there can be progression to central precocious puberty (4). Premature adrenarche or premature pubarche is characterized by elevated androgens, namely dehyroepiandrosterone (DHEA) or its sulfate (DHEA-S), and isolated androgenic pubertal changes such as pubic and axillary hair growth, body odor, or acne (8). Isolated premature menarche is sometimes also believed to occur (3).

Central precocious puberty (CPP), also known as true precocious puberty, involves the activation of the HPG axis and is thus gonadotropin-dependent. It is usually characterized by breast development with or without pubic hair growth, rapid progression of puberty, elevated gonadotropin levels, and an accelerated linear growth rate (8). When CPP is idiopathic, pubertal development follows the normal pattern but differs primarily in the fact that it occurs at an earlier age. However, a number of pathological conditions may be responsible for CPP, most notably abnormalities of the central nervous system (CNS). These include congenital neurological and CNS problems such as hydrocephalus or hypothalamic hamartomas; tumors and lesions such as gliomas, astrocytomas, or cysts of the pineal or pituitary glands; neurological insults resulting from infection-related inflammation such as encephalitis or meningitis; and CNS trauma or other insults from medical treatment such as radiation, chemotherapy and surgery (3-5). CPP can also result from longstanding peripheral precocious puberty (9).

In the case of peripheral precocious puberty or pseudoprecocious puberty (PPP), one might see rapid progression and/or multiple pubertal signs without the involvement of gonadotropins typical of CPP. Some causes of PPP include sex steroid production from tumors of the adrenal glands or ovaries in females as well as estrogen secretion by ovarian cysts. In some cases, persistent hypothyroidism can also be associated with PPP due to the binding of thyroid-stimulating hormone (TSH) by the FSH receptor (9). Genetic mutations can also lead to PPP. One such condition caused by a G protein mutation is the McCune-Albright syndrome (MAS) which leads to skin discoloration, polyostotic fibrous dysplasia of the bones, and ovarian estrogen secretion leading to precocious puberty (8). Congenital adrenal hyperplasia (CAH) is caused by a different

mutation and leads to excessive androgen production that can cause signs of adrenarche and bone maturation as well as genital masculinization (8).

More specific categories of early pubertal development have also been named, such as the thelarche variant or variant thelarche used to describe patients older than the typical infants and toddlers with premature thelarche or those who have more progressive forms of breast development but do not quite fit the criteria for CPP with advanced growth (10). Similarly, patients who may have both estrogen- and androgen-related pubertal changes but limited or no pubertal progression have often been labeled with slowly progressing, unsustained, or nonprogressive precocious puberty in the literature (11), which is sometimes considered a variant of CPP (12) and could reflect sporadic activation of the HPG axis (5).

Statistics on precocious puberty reveal that it is up to 10 times more common in girls (7). Research has shown that around 20% of cases presenting with a complete form of precocious puberty are peripheral, with the remaining majority being of central origin (13). CPP in girls is predominantly idiopathic, with some estimates indicating that no cause can be found in up to 85% of cases (5); and particularly in girls with CPP who are six years of age or older, the proportion with CNS lesions was found to be around only 2% according to one study (14). For about a quarter of girls with CPP, the condition runs in families (3).

1.2 Assessment of Precocious Puberty

A typical evaluation for precocious puberty to distinguish between the subtypes mentioned above would include a physical examination, an assessment of the patient's medical history and family history, laboratory testing, and possible medical imaging (9). The level of advancement of clinical pubertal signs—breast development and pubic hair in particular—are assessed using Tanner stages, which consists of a scale of 1 to 5 measuring pubertal progression, with 1 representing the prepubertal stage. The vaginal mucosa can also be examined to check for estrogenization, characterized by pink rather than red mucosa as well as discharge, and to ensure that the clitoris is not enlarged, which could point to a condition such as CAH (4). Both rapid progression of pubertal features over time and an accelerated linear growth rate are more indicative of true precocity, while the benign conditions of premature thelarche and premature adrenarche are typically associated with normal growth rates. In addition, a bone age read from an x-ray of the left hand that demonstrates skeletal maturation greater than two standard deviations above the chronological age is considered advanced and likewise more indicative of true precocious puberty (5).

Although there is no set cutoff for hormone levels to diagnose subtypes of precocious puberty and there is some overlap between pubertal and prepubertal levels in some cases (9), cutoffs ranging from greater than 0.3 IU/L to greater than 0.6 IU/L for a random LH level have typically been suggested in the literature (15-16). The administration of a GnRH stimulation test is also useful in differentiating CPP from other forms of early puberty since a pubertal response will be observed in the case of CPP

whereas gonadotropin levels will remain low in puberty that is gonadotropin-independent (8). In general, a pubertal response also usually has greater elevations in LH while a prepubertal profile and response is dominated by FSH (9). Suggested cutoffs between stimulated prepubertal and pubertal LH values range from 3.5 IU/L to 8 IU/L or greater (16-17).

A pubertal estradiol level above 20 pg/ml may also point to CPP, but in the absence of pubertal gonadotropins is indicative of PPP (8). Premature adrenarche and premature thelarche also have prepubertal gonadotropin levels, or raised FSH levels in some instances of thelarche (8), with generally little to no elevation in other sex steroids such as estradiol and testosterone; but DHEA or DHEA-S levels are usually in the pubertal range in the case of premature adrenarche (5). In addition to DHEA-S, testing for elevation of 17-hydroxyprogesterone and testosterone can be used to rule out CAH (8).

A pelvic ultrasound may also be indicated to look for ovarian cysts or tumors and aid in the diagnosis of the child presenting with early puberty, as enlarged ovaries and uterus may be present in a girl with CPP compared to one with premature thelarche; and the presence of endometrial development also indicates estrogen activity. Magnetic resonance imaging (MRI) of the brain can also be useful to check for CNS abnormalities as the cause of precocious puberty if this is suspected (9).

1.3 Management of Precocious Puberty

In the case of a tumor being the underlying cause of precocious puberty, its removal can effectively treat pubertal development (4). In other situations, treatment with GnRH agonists (GnRHa) can be used to halt the progression of puberty and delay menarche and may even lead to regression of breast tissue in some instances (9). GnRHa therapy is only advised for children with progressive CPP. One of the other main benefits of treatment is the slowing of the linear growth rate, as one of the primary concerns in CPP is compromised adult height due to an early pubertal growth spurt and rapid advancement of skeletal maturation ahead of actual age (17). However, those without progression or with incomplete forms of precocious puberty are not at risk for a decreased height. Thus, treatment is not needed in patients with incomplete or slowly progressive precocious puberty (16). Moreover, studies have shown that for pubertal onset over the age of 5 or treatment over the age of 6, there is little benefit in terms of height from GnRHa therapy (17). Yet, other reasons that may be considered in determining if treatment is appropriate have included concerns that the child may not be psychologically prepared to handle puberty and menses because of age or especially if the child is developmentally delayed (17).

Several options exist for GnRHa treatment, the most widely used being monthly injections of depot leuprolide; but other therapies may be in the form of daily nasal sprays or annual implants (5). In patients with PPP, GnRHa therapy is not useful because their puberty is not dependent on gonadotropins. Rather, treatment of the underlying cause is needed. For instance, patients with MAS can benefit from estrogen blockers such

as tamoxifen, and cortisol can be administered in some patients with CAH or cortisone deficiency (3). Studies have also shown that treating girls who have premature adrenarche with metformin can postpone early menarche and increase height (18).

Although treatment with GnRHa is relatively safe and effective, there has been some concern over weight gain and decreased bone mineral density as a result of it, but the evidence does not appear to support increased weight with treatment or long-term effects on bone mineral density (17). In fact, a recent study in Korea did not find an increase in obesity or detrimental effects on bone density in girls assessed with DEXA scan after one year of treatment with GnRHa (19). However, other side effects of treatment include the development of abscesses at the site of injection or implantation (20). In addition, an increased risk of polycystic ovarian syndrome (PCOS) has been demonstrated in girls treated with GnRHa (21).

1.4 Pubertal Timing in US girls

Evidence suggests that there has been a trend towards earlier puberty in girls in the United States. With regards to menarche, historical records indicate that the average age at which girls reached menarche declined from 17 years in the mid-1800s to less than 14 years by the mid-1900s (13), and this change was largely a result of better nutrition and health status (2). Analyses of more recent data indicate that the median age at menarche dropped about 2.5 to 4 months in the 25 to 30 years between the National Health Examination Survey (NHES) cycles I, II, and III (1960-1970) and the National Health and Nutrition Examination Survey (NHANES) III (1988-1994) (22-23); however,

a few studies also report that the decline in age at menarche has not been consistent because an increase during some time periods, particularly for those born in the 1960s compared to previous decades, has been demonstrated (24). Some researchers have asserted that the age at menarche is now leveling off or has already done so (13, 24-25), although analyses continue to demonstrate some decline, albeit less drastic than previously recorded (26). Analysis of NHANES 1999-2002 data for girls aged 9-15 years places the current overall mean age of menarche at 12.34 (27).

Studies of age at menarche have also demonstrated racial differences in the average age as well as in the apparent secular trend. Studies consistently show an earlier average age of menarche for black girls in comparison to white girls based on data since the 1960s (28). More recently analyzed mean ages of menarche from the continuous NHANES 1999-2002 are 12.52 years for white girls and 12.06 years for black girls (26). Furthermore, analyses of menarche data over time indicate that the rate of decline in age at menarche is not the same across racial groups. The Freedman et al. (29) analysis of data from the Bogalusa Heart Study conducted between 1973 and 1994 found a 2 month drop in median age of menarche among white girls and a 9.5 month drop among black girls over the course of the 20 years of the study. However, the Louisiana population in the study was not representative of the general US population (13). The Chumlea et al. (23) comparison of NHES and NHANES III data found a more modest difference in the change in menarcheal age by race, with a decline of a quarter of a year, or about 3 months, in white girls and about 5.5 months in black girls in the 30 years between the two studies. Racially differing trends are also supported by a study by McDowell et al. (30) in which women surveyed in the continuous NHANES cycles from 1999 to 2004 were

divided into birth cohorts of ten year increments, and this study showed that it was not until the 1950-1959 birth cohort that black girls began to have an earlier average age at menarche than white girls. Prior to that time, black girls actually had a later age at menarche than their white counterparts. The drop in menarcheal age between women born before 1920 and those born in the latest cohort analyzed in the study, 1980-1984, was 0.8 years for whites and 1.4 years for blacks.

Examination of secondary sexual characteristics similarly illustrates a trend towards earlier development along with racial differences. There is little early data on secondary sexual characteristics compared to menarche; however, a few small studies done from the 1930s to the early 1970s placed the average age at onset of breast development around 11 years (31). Thus, when the first large-scale study capable of assessing onset of breast development—the Pediatric Research in Office Settings (PROS) study of 17, 077 girls conducted in 1992 and 1993—reported a much earlier mean age of 9.96 for white girls and 8.87 for black girls (32), the results were surprising (31). Moreover, the study found that 10.5% of white girls and 37.8% of black girls had some breast development by 8 years of age. Yet, because the study was clinic-based and consisted of a convenience sample, the results were not truly generalizable to the overall US population (2). When the NHANES III data was analyzed, the mean age for breast development was 10.3 years and 9.5 years for white and black girls, respectively, with 11.4% of white girls and 27.8% of black girls having some breast development at 8 years of age (33). A separate analysis by Sun et al. (34) produced similar results—a median age of 10.38 years for whites and 9.48 years in blacks. Although these figures are not as early as those reported in the PROS study, they nevertheless represent a decline from what was

previously thought to be the onset age for breast development. More recently, another study seems to indicate further increases in early breast development. Although the study involved 1239 girls from just three US sites, it found that by age 8, the prevalence of breast development was 18.3% for white girls and 42.9% for black girls, which is markedly greater than in past studies (35).

Less focus has been placed on pubic hair development, but a racial gradient exists there as well. The mean ages reported from one NHANES III analysis were 10.5 years and 9.5 years for white and black girls, respectively (33). Earlier reports produced average ages ranging from 11.0 to 11.9 years; but, again, these were based on very small studies (31). Nonetheless, it appears that there has been a decline of about 6 months in the average age of onset of pubic hair development (2).

In conclusion, the general agreement among experts is that there has been a secular trend towards earlier onset of puberty in US girls, which is most pronounced for breast development but also exists to a lesser extent for menarche; and data is more limited and less conclusive for pubic hair development as well as for changes in the rate of progression through puberty (2). While some data exists on pubertal development in Mexican American and Hispanic populations in the US, it is excluded for the purposes of this study which focuses solely on white and black girls.

1.5 Factors Influencing the Timing of Puberty

The data confirming secular trends as well as racial differences in pubertal timing in girls has sparked further investigation into factors affecting pubertal onset. Genetics is

one factor that plays a substantial role in pubertal timing, and about 50% of the variation in age at menarche has been found to be attributable to genes (36). Recent work has identified a number of genes involved in the regulation and timing of puberty (3, 37-40). On a population level, genetics has been considered as a possible factor in racial and ethnic variations in pubertal timing in the US and around the world, and some studies have pointed to regional and ethnic differences in age at menarche; but the inability to exclude environmental factors in these studies in addition to inconsistencies in results among migrating populations have made it difficult to obtain conclusive evidence on genetic reasons for regional and ethnic differences (13).

Moreover, in terms of genetic explanations for racial and ethnic differences in pubertal timing in the US, the recent origin of the racial disparity in age at menarche further complicates purely genetic claims. It is also important to note that many studies on African girls point to a later age at menarche compared to American blacks and Americans in general, although African countries are also documenting secular trends (41-43). While the apparent later age of menarche in Africa may be related to differences in nutritional and socioeconomic status (SES), some studies among well-to-do African populations still report menarcheal ages much later than African Americans (13); however, one study in Cameroon found that a subset of wealthier girls had a mean menarcheal age of 12.72, one of the lowest reported from African data and one that the authors claim is approaching the age in industrialized countries (44). Not as much data is available on secondary sexual characteristics, but recent data from urban South Africa reported a median age of breast development of 10.1 years among blacks and 10.3 years for pubic hair growth, with a menarcheal age of 12.4 (43, 45). These results do not differ

from those found in white South Africans (10.2, 10.5, and 12.5 for breast development, pubic hair, and menarche, respectively) and were considered comparable to United States figures for menarche and breast development overall. However, the author does not mention whether these numbers differ significantly from the race-specific ages in the United States; and considering that the majority of African slaves originated from West and Central Africa (46), comparisons with South Africa may not be appropriate.

Nevertheless, the fact that a secular trend has been observed in the US—as well as evidence that earlier menarche in blacks compared to whites is a recent development from the latter half of the 20th century—points to a significant contribution of environmental factors to pubertal timing. Therefore, a combination of many factors including genes, societal factors, and the environment is likely to be responsible for earlier puberty and racial differences (2).

The most commonly discussed influence on pubertal timing appears to be adiposity and obesity. Usually measured as body mass index (BMI), numerous studies have demonstrated an association between high BMI and earlier age at menarche, thelarche, and pubarche. For example, Anderson et al. (22) found that girls who reached menarche earlier in both the NHES and NHANES III had a higher BMI. In addition, the analysis of NHANES III by Rosenfield et al. (47) demonstrated that from age 8, heavier girls were significantly more likely to have started breast and pubic hair development. However, because most of the studies are cross-sectional, it is not possible to conclude a causal link between greater weight or fat composition and early puberty, and this is especially complicated by the fact that puberty itself also leads to greater fat accumulation (31). Nonetheless, other studies have demonstrated a similar association

between BMI in early childhood prior to the onset of puberty, indicating that the association is likely causal (48). For example, a prospective study on young girls showed that greater BMI at 3 years of age predicted earlier puberty (49), and other studies have linked marked weight increase through 9 months of age (50) and rapid growth and weight gain in early childhood (51) to early menarche. Greater meat intake in particular during childhood has been demonstrated to have an association with earlier menarche (52).

Thus, due to an increasing prevalence of obesity in the US and among blacks in particular, it has been postulated that this may be behind the secular trend towards earlier puberty and its racial disparities. For example, in the NHES for the years 1963-1965, BMI greater than 95% had a prevalence of around 5% in both black and white girls aged 6-11; but by NHANES 1999-2000, the prevalence was 11.6% for white girls and 22.2% for black girls (8). This racial disparity in weight may be partly attributable to the racial segregation of residential environments that exist in the US and unequal access to resources, as it has been shown that parks and recreational facilities that can promote physical activity to protect against obesity are significantly fewer in areas concentrated with minorities and lower SES (53). Similarly, these areas have been found to have significantly more fast food restaurants and limited access to supermarkets and fresh produce, which are factors that have been linked to obesity rates (54).

Yet, when weight status has been considered in analyses of puberty data, even among only those with normal BMI, black girls still had significantly greater pubertal advancement than their white counterparts (47), and menarche was still earlier among black girls when controlling for weight (22). Moreover, the association between BMI and puberty onset was found to be weaker among black girls based on the PROS study (55),

although for menarche, Gonzalez-Feliciano et al. (56) found a stronger relationship with BMI among blacks than among whites in their stratified analysis, and Wu et al. (33) saw a loss of significance in the racial differences for menarche—but not for secondary sexual characteristics—when controlling for BMI in their analysis of NHANES III data. Thus, while obesity may play a part in the racial differences in pubertal timing, it does not appear to account for them.

Related to the idea of obesity is the association between physical activity itself and pubertal timing, as it has long been known that excessive exercise and thinness can delay puberty, particularly menarche (48). Another proposed mechanism relates to leptin levels. Leptin is a hormone that plays a role in the storage of fat and has been found in higher levels among black girls in comparison to whites; and some studies have shown associations between leptin and puberty timing independently of BMI and fat (1, 8). Moreover, others have demonstrated increasing levels of leptin in girls prior to the onset of puberty (48). Likewise, the hormone insulin has been implicated in studies on pubertal timing because of higher rates of insulin resistance among African Americans, along with the observed association between hyperinsulinemia and premature adrenarche and girls with early puberty in general (48).

Low birth weight or small size for gestational age is another factor suggested to be linked to earlier maturation (7). One study found that babies who were thin in combination with having a greater birth length attained menarche about half a year earlier than shorter babies of similar weight and that the effects of birth size were heightened if there was also rapid growth in the first months of life (57). However, the results on birth size in relation to pubertal timing have not always been consistent (2).

Psychosocial factors have additionally been suspected to affect the timing of puberty. In particular, stressful living conditions related to factors such as sexual or physical abuse, harsh maternal parenting, low SES, and single parent households where no father is present have been linked to earlier pubertal timing (7, 58-61). Some theories refer to evolutionary advantages of being able to reproduce early and carry on one's genes in stressful environments (59), although other extreme stressful situations such as war are known to be associated with delayed puberty (13). In addition, not only is the absence of one's father presumed to affect pubertal timing, but, in particular, the presence of a stepfather or unrelated male may also be associated with earlier puberty in girls; and stress associated with these situations in addition to pheromones are hypothesized explanations for this possible link (7, 25).

A phenomenon of precocious puberty among girls adopted from foreign countries has also been observed in a number of European countries, and proposed reasons for this relate to many of the same issues mentioned above, such as nutritional improvements and catch-up growth upon moving after small size at birth, previous stressful living conditions or the stress of adoption, living with unrelated family members, as well as previous environmental exposures (7, 62). For instance, some studies have found that children who migrated with their families had a lesser tendency towards early puberty as those who were adopted, which may provide support to theories regarding stress and exposure to unrelated males but does not rule out nutritional explanations (63-64).

There is a differential in many of the possible etiologic factors by race, such as higher prevalence among African Americans of low SES, low birth weight, and single-parent households. For instance, 44% of black participants compared to 11% of white

participants in a study on father absence and pubertal onset were living without a father (61). Additionally, African Americans are about twice as likely to have low birth weight as white infants; and researchers have explored the contribution of stress, including stress associated with racism, and its physiologic effects in understanding this phenomenon, with fairly suggestive results (65). SES has also been consistently associated with low birth weight and related outcomes, both on the individual and community level (66); thus, low SES and the undesirable neighborhood characteristics found in low income neighborhoods may be an additional mechanism for racial differences in birth weight, given racial disparities in SES and neighborhood and living conditions that exist in this country. Further study is needed to investigate the importance of disparities in such factors in explaining race differences in pubertal development.

Another proposed mechanism for earlier puberty relates to light exposure, as earlier age at menarche has been observed in girls who do not have sight in addition to evidence of seasonal variations in menarche (13). In particular, the hormone melatonin—which is secreted by the pituitary gland that helps to regulate puberty—is believed to play some role in pubertal onset and progress since melatonin levels drop during puberty (5). As a result, some scientists have suggested that modern lifestyle and its associated technologies, including activities such as watching television at night, could exacerbate the early onset of puberty by preventing melatonin secretion which requires darkness (67). Results from a recent pilot study may support the melatonin hypothesis, as girls in the study who had precocious puberty were found to have the lowest blood melatonin levels, followed by girls going through normal puberty and, lastly, prepubescent girls with the highest levels (68).

Of growing concern is the possible role of chemical exposures—both natural and synthetic—in promoting earlier puberty. For example, direct exposure to hormones through meat and dairy products was suspected as a reason behind a few cases of wide-spread early puberty incidents in various countries (8). In addition, exposure to the naturally occurring plant estrogens in soy called phytoestrogens particularly early in life, such as through baby formula, is also being considered as a possible factor interfering with the endocrine system and thus affecting pubertal timing. Moreover, the fact that a higher proportion of African Americans are fed soy formula due to higher rates of lactose intolerance among them has led to some speculation about its role in the racial differences in pubertal onset (7). However, human data on the link between phytoestrogens and puberty is lacking (69)—though a significant association between soy formula use and breast development in two-year olds has been found in one study; but an association was not observed during children's first year of life, which the authors attribute to the already present elevated estrogen levels related to the initially activated HPG axis in early infancy (70). On the other hand, in another study of older children, urinary levels of the phytoestrogens daidzein and genistein were higher in 9 year old girls with no breast development compared to those with development (71), and a more recent study also demonstrated a similar though weak inverse association (72).

Another frequently mentioned source of direct exposure to hormones is cosmetics, especially hair care products. Some personal care products (PCPs) have been found to contain hormones such as estrogen or to contain placenta, which is also high in hormones; and cases have been reported of young children developing breasts or beginning to menstruate after using these products, with subsequent subsiding of their

pubertal development after termination of use (73). Furthermore, several studies have documented much greater use of these hormone-containing hair products (HCHPs) among African Americans than other racial groups (74-77).

There have also been isolated cases of babies conceived through assisted reproductive technologies (ART) showing signs of puberty at birth presumably due to their mothers' use of hormones in the treatment process; however further research is needed on ART use and subsequent puberty of the child (7).

Research has also been advancing into exposure to endocrine disrupting chemicals (EDCs) that have estrogen-like effects and could affect the timing of puberty—such as phthalates, which are used in plastics, pesticides like dichlorodiphenyltrichloroethane (DDT), and brominated flame retardants. For example, in the first study linking environmental exposures to early puberty, the flame retardant PBB was shown to be associated with earlier menarche (78); and several other studies have since found associations between different chemicals and altered pubertal timing—both early and late (79-82). Children and women have been found to have higher body burdens of certain EDCs, and there is also some indication that levels of some EDCs vary by race, as greater levels of bisphenol A (BPA) and metabolites of phthalates were found in the urine of African Americans (83-84). However, firm conclusions on EDCs' influence on pubertal timing and on racial disparities cannot be drawn from the available evidence, as many EDCs have varying effects. For instance, a recent study on phthalates did not find an association with precocious puberty (85) while another study on girls from the Breast Cancer and Environmental Research Centers (BCERC) cohort found positive relationships between levels of phthalate metabolites with low molecular weight and both

breast and pubic hair development but an inverse relationship for metabolites of high molecular weight and pubic hair; but these results were weak or borderline significant at best (72).

PCPs are again a reason for concern because they often contain EDCs as well. For instance, phthalates have been found in high prevalence in many PCPs (86). Additionally, attempts have been made to link the controversial mercury compound thimerosal to early puberty (87), and other metals have also been studied in relation to pubertal timing (79).

1.6 Disagreements over Pubertal Timing—Normal and Abnormal

Although the general consensus appears to be an acknowledgment of a secular trend towards earlier puberty, some scientists in the field still maintain that a secular trend cannot be concluded from the available data (2). Reasons cited for this relate to the quality of pubertal onset data, namely concern over the appropriateness of the age ranges of the study populations and whether they adequately covered possible ages of onset; inadequate sample size in early studies; generalizability in studies such as PROS and Bogalusa that were not based on nationally representative samples; and, even among the national surveys, accuracy of the methods of ascertainment of pubertal features —such as the fact that measurement of breast development was often by inspection only as opposed to palpation of breast tissue. Furthermore, the use of different statistical techniques to generate the estimates among the studies has limited their comparability.

Nonetheless, the majority of researchers are in agreement with regards to changes in pubertal timing, but what has rather become even more controversial is the suggestion

in 1999 by members of the Lawson Wilkins Pediatric Endocrine Society to lower the age cutoff for precocious puberty in girls from 8 years to 7 years for whites and 6 years for blacks based on the PROS study results published 2 years prior that demonstrated a high prevalence of early development, as well as the fact that the original age criterion was based on British findings (88). In fact, many of the studies on pubertal development that followed the PROS study were carried out in response to this recommendation (28). Concerns over the proposed age limits again point to the data quality issues mentioned above—many of which are applicable to the PROS study on which the new guidelines are based—and also the fact that the new recommendations would mean delaying treatment in girls that may benefit from starting therapy earlier (8). Most importantly, there is a concern that the revised age limits for precocious puberty would result in overlooking some patients with true abnormalities and pathological causes for their sexual precocity who require evaluation and intervention (89). Therefore, the recommendations have not been universally adopted by physicians (8).

The proponents of the revised recommendations have responded to these claims with evidence pointing to the fact that many girls with early development in the age range in question do not go on to suffer adverse effects and do not receive much benefit from treatment in terms of adult height in particular; moreover, most 6 to 8 year old girls with early puberty do not have CNS abnormalities (8).

1.7 Consequences of Early Puberty

Regardless of the controversy surrounding secular trends and a revision of the precocious puberty age cutoffs, as previously mentioned, it is recognized that unusually early puberty can result in reduced final adult height due to the fact that particularly young children undergoing puberty may have an advanced bone age relative to their actual age and will stop growing earlier (7). In addition, early menarcheal age is associated with a greater risk of breast cancer, and there is some indication of an association with ovarian cancer as well (24). Premature adrenarche in particular has also been shown to be associated with PCOS later in life (90). There have also been links to other non-reproductive health outcomes. For instance, an association between early menarche and higher mortality has been demonstrated in a Norwegian cohort after adjusting for BMI, occupation, and other factors; though smoking and exercise could not be controlled for (91). However, a similar association in a US cohort has subsequently been demonstrated for overall as well as stroke- and heart-related mortality (92). A couple of studies have also found an association between early menarche and future diagnosis with asthma, most recently a study in girls in Canada (93). There may also be some connection between early puberty and metabolic syndrome, diabetes, and adult BMI that need further exploration as results have not been consistent (24).

Early puberty also has psychological and behavioral effects on the child or adolescent based on its association with earlier sexual initiation, increased substance abuse, poor behavior in school, criminal activity, negative body image, depression, and other forms of mental distress (25, 94-95). Although many of the behavioral effects do

not last into adulthood, women who had early menarche have been known to be less educated and work in lower-tier jobs than their later maturing counterparts (3); and associations with depression and a greater number of sex partners have also been shown to continue into early adulthood according to a recent study (96).

1.8 Study Aims & Significance

Apart from studies examining racial differences in pubertal timing in the US population, few studies have truly characterized precocious puberty patients, let alone racial differences in precocious puberty patients, and describing the condition is a necessary first step in identifying etiologic factors for the racial differences and trends in pubertal onset. Both Midyett et al. (89) and Kaplowitz (97) did study a population of precocious puberty patients in which the race of patients within subtypes was documented, but in neither study were any laboratory results analyzed nor were direct comparisons of other parameters by race made, apart from an analysis of BMI and growth data in the Midyett et al. study. An earlier, small study limited to 20 patients with unsustained or slowly progressive precocious puberty also noted the race of the patients but did no comparisons of any characteristics by race (11). Nor have studies in European populations included race. For example, a recent analysis of over 400 girls evaluated for precocious puberty in Denmark was limited to Caucasians (98).

Thus, this study aims to utilize medical records of girls with precocious puberty to differentiate between types of precocious puberty and evaluate whether various parameters differ between African American and Caucasian girls. Better knowledge of

how precocious puberty differs by race can improve our understanding of the distribution of precocious puberty in our population and thus facilitate diagnosis and management of the condition. In addition, it will contribute to the ongoing debate over race-based guidelines for diagnosing precocious puberty. Furthermore, because many environmental factors are purported to be related to changes in pubertal timing and exposure to them is not considered beneficial, it is critical to clarify racial variations in precocious puberty in order to elucidate the role of the environment and its differential effects by race so that the issue of early puberty can be better addressed and the associated exposures and their adverse effects mitigated. By illuminating racial variations in the characteristics of girls with precocious puberty, this study can therefore guide future research evaluating environmental exposures for associations with precocious puberty and for racial differences in exposure.

Chapter 2: Methods

2.1 Study Population

This study was reviewed by the Institutional Review Board (IRB) of Emory University and received expedited approval with a complete Health Insurance Portability and Accountability Act (HIPAA) waiver, waiver of informed consent, and waiver of assent.

A retrospective chart review was conducted using patients from Emory Children's Center (ECC) Division of Pediatric Endocrinology and Diabetes. In June of 2010, patients evaluated for precocious puberty were identified from the ECC billing records by *International Classification of Diseases, Ninth Revision* (ICD-9) code for precocious puberty/thelarche/adrenarche/menarche, 259.1, for visits in the most recent two year time period from May 1, 2008 through May 30, 2010. This produced an initial list of 390 unique patients. Charts were pulled from the list by medical records staff in numerical order according to the two terminal digits of the patient medical record number (MRN). Charts were examined for race, date of initial visit, age, and confirmation of diagnosis prior to full review. Abstraction from patient charts was eventually limited to female patients identified as white/Caucasian or black/African American as recorded in the chart by the medical provider or in previous records from the referring physician, or based on the author's assessment of a patient photograph, when included, if race was not explicitly indicated. An additional patient's race was determined as black based on statements describing the parents to have originated from West Africa. Those patients only identified

as Hispanic or Latin American—which is considered an ethnic category as opposed to a racial category—were excluded from the study since race was not additionally included. Patients identified as biracial were also excluded. Therefore the study subjects approximate a sample of non-Hispanic white and non-Hispanic black patients.

Patients were included in the study sample whose initial consultation for precocious puberty fell within the 4-year time frame between March 29, 2006 and March 28, 2010. Four years was determined to be an appropriate time frame to obtain an adequate number of patients and patients with sufficient follow-up evaluations, and the selected dates represented the most recent four years allowable as a retrospective study by IRB based on the date on which the IRB application was initiated (March 29, 2010). Thus, patients whose initial visit occurred after March 28, 2010 were excluded from the study, and for those patients included, follow-up visits occurring after March 28, 2010 were not reviewed or utilized for abstraction of data. Patients who had follow-up visits after March 29, 2006 but whose initial visit at ECC preceded this date were likewise excluded from the chart review. For purposes of consistency with patients seen solely at ECC, patients whose initial visit at ECC was within the time frame but who had been previously evaluated for and diagnosed with a form of precocious puberty by another endocrinologist before the study start date were similarly excluded. In other words, a visit regarding puberty with any endocrinologist prior to March 29, 2006 was cause for exclusion. Therefore, the study sample contains incident as opposed to prevalent diagnoses—although actual puberty onset may not have been incident.

Charts were reviewed as available starting at the beginning of the billing list until 50 patients of each race who qualified for inclusion could be obtained. Listed patients

whose charts had been moved to offsite storage were ordered and delivered for review once available charts had been examined, and charts that were unavailable during the initial run-through of the list were requested again. Therefore, all charts on the initial list were examined to identify the charts for full review, apart from 11 patients who had no or incomplete MRNs as well as 18 charts that could not be located at the time and 1 chart that did not belong to the endocrinology division. This resulted in a total of 360 charts examined from the list, of which 50 were excluded because of endocrinologist evaluations for precocious puberty outside of the study date range or a lack of complete records indicating when their evaluation began in relation to the dates included in the study; 134 were excluded because of the absence of race information, unclear race based on photograph or chart descriptions, or race other than black or white; and 17 were excluded because a diagnosis related to precocious puberty was not confirmed or could not be determined given incompleteness of the records. An additional 40 were excluded for a combination of the above reasons, and 3 patients were also excluded who had male sex. An additional 17 charts were excluded for black race that were otherwise eligible when the target sample size for black patients had been obtained and only white patients were needed. The total number of exclusions for all above reasons thus totaled 261. The final sample therefore contained a total of 99 patients, 49 of whom were white (including 1 identified as being of Middle Eastern origin) and 50 of whom were black.

2.2 Data Collection and Measurements

An abstraction form was created as an electronic database using Epi Info 3.5.1—a software package produced by the Centers for Disease Control and Prevention (CDC)—into which data was entered directly using a laptop. The abstraction form included several variables relating to patient demographics and social history, birth history and development, medical history, family history, clinical and laboratory parameters relating to precocious puberty, and treatment information. This data was abstracted from all sections of the chart including patient registration and billing information, outside records from referring physicians, radiology and laboratory reports, notes and correspondence, and initial and follow-up visit reports. For the complete abstraction form, see Appendix A. Only a selection of these variables was used in the current analysis, and these are described below.

2.2.1 Diagnosis

Patients' initial diagnoses were determined for analysis and were based on the assessment and impression of an endocrinologist at ECC or elsewhere from the initial visit or after confirmation of a diagnosis if testing and follow-up was needed to rule out differential diagnoses. Impressions made by other medical professionals who were not specialized in endocrinology, such as primary care physicians, were not considered official diagnoses. In cases where a patient was evaluated at multiple locations and the diagnoses conflicted (n=3), the opinion of ECC took precedence to maintain consistency.

An ECC endocrinology fellow was also consulted to clarify information in the charts when needed. The following categories were then used to classify patients taking into consideration doctors' remarks and patients' clinical and laboratory characteristics:

- *Premature adrenarche (PA):* Isolated sexual hair (pubic or axillary hair) and/or an associated elevation in androgens.
- *Premature thelarche (PA):* Isolated breast development without other physical and biochemical evidence of CPP.
- *Central Precocious Puberty (CPP):* Breast development, with or without the presence of adrenarche, in addition to other indications of true central puberty (i.e. quickly progressing pubertal development, pubertal linear growth spurt, advanced bone age, elevated gonadotropins, or menarche).
- *Other Precocious Puberty (OPP):* Thelarche in addition to adrenarche that were not judged to be the result of true CPP because of a lack of or failure to confirm other signs consistent with true CPP; or pubertal signs that were occasionally labeled as a combination of premature thelarche and premature adrenarche. These patients may be comparable to those identified as slowly, unsustained, or nonprogressive precocious puberty in the literature (8-9, 11-12). This category also included 1 patient with PPP caused by an ovarian cyst.

2.2.2 Etiologies & Comorbidites

Other medical conditions were taken from the patient medical history information, and patients were classified as having an etiologic pathology or comorbidity

if conditions known to cause precocious puberty or other relevant conditions of interest associated with precocious puberty were present. These included CNS lesions, any other condition resulting in compromised neurologic function, or endocrinopathies. Other significant disabilities or abnormalities were also noted.

2.2.3 Body Mass Index

BMI was expressed as a percentile and z-score based on the 2000 CDC growth charts for the US given the patient's age and female gender. This was derived from the height and weight at the initial visit with ECC and the age in months corresponding to the date at initial visit in relation to patient date of birth. Three height readings in centimeters were taken and recorded in the notes for each patient visit at ECC, and an overall or final height was typically reported by the endocrinologist in the dictated letter from the initial visit. This overall height was based on the most common measurement (i.e. when two of the three readings were the same if all three are not identical) or the average or middle value if all three differed. Thus, the final value out of three readings that was reported by the doctor was selected for the BMI calculation, unless the reported value did not correspond to this convention or a clear error in the dictation was observed, no dictated report was present, or no final height was selected (n=8); in which case, the height was chosen from the 3 readings in accordance with the same general method (i.e. most common or average reading). Patient weight was recorded in kilograms, with the exception of 1 patient for whom it was recorded in pounds and thus converted to kilograms.

Although BMI was included in a portion of the patient charts, all patients' BMI was calculated using the SAS program code provided by the Centers for Disease Control and Prevention (CDC) based on the 2000 CDC growth charts for the US to ensure consistency.[i] BMI was not calculated on infants and measures based on length as opposed to stature, in accordance with the CDC program. Although BMI growth charts commence at 2 years and height can be taken for children from this age, all children under 36 months were assumed to have measures of length rather than stature for conservativeness based on the fact that CDC infant growth charts using length can be used on children up to 36 months if the child is unable to stand stably without assistance.[ii] Therefore, 15 patients had recumbent height measures based on this criterion (11 black, 4 white). Of these 15 patients, 12 were under 2 years (9 black, 3 white), and the remaining 3 were under 3 years (2 black, 1 white). Two additional black patients did not have a height reading due to physical disability and inability to stand. Thus, BMI was calculated on a total of 45 white patients and 37 black patients.

2.2.4 Income Level

Income level of the patient's family was approximated by the type of medical insurance held by the patient, namely whether or not the patient had any coverage through Medicaid or PeachCare for Kids at any point in time during their evaluation. PeachCare for Kids is a program in Georgia available to children from families who do not qualify for Medicaid but whose income is no more than 235% of the poverty level.[iii]

The income limits for Medicaid qualification for children depend on the family size and child age for the specific coverage.[iv]

2.2.5 Age

Age at initial evaluation for precocious puberty was calculated in years from the number of days between the date of birth and the earlier of either the date of diagnosis—if initial endocrinologist evaluation was not at ECC (n=7)—or the date at initial visit at ECC divided by 365.25 days. One patient had been followed by ECC endocrinologists for another reason before signs of puberty commenced, so the age was based on the visit in which puberty was first noted. For another patient, the day of evaluation was not available because she was seen at an outside clinic and the record was not included in the ECC chart. Thus, based on the date of follow-up and time interval specified since the initial evaluation, and given the month and year for the initial evaluation, the day was approximated by subtraction of the time interval in days from the date of follow-up.

2.2.6 Estradiol

The earliest estradiol laboratory result available for each patient was selected, and the age in years associated with the date of the laboratory test was calculated in the same manner as described for age at initial evaluation above. All estradiol levels were converted to picograms per milliliter (pg/ml), if not already reported as such. For one patient who had a repeat analysis of her laboratory result, the lower of the two values for

the test was taken as a conservative measure. Results that were below a limit of detection (LOD) were replaced with the value of the respective LOD divided by the square root of 2 for the analysis.

2.3 Statistical Analysis

The percentage of patients within each diagnosis category was calculated for each race, and a chi-square (χ^2) test was used to determine whether the distribution of subtypes differed between the races. χ^2 tests or Fisher's exact tests—if expected cell counts were less than 5—were also used to test for significant differences by race and diagnosis in the proportion of patients classified as overweight (BMI $\geq$ the 85th percentile[v]) or obese (BMI $\geq$ 95th percentile[v]). Income level was dichotomized, with low income defined as ever being on Medicaid or PeachCare for Kids, and percentages of patients from low income backgrounds by race and diagnosis were generated and also tested with χ^2or Fisher's exact tests, along with the calculation of a risk ratio for the strength of the association.

For each race overall and within each diagnosis category, univariate analyses producing frequency distributions and summary statistics including means, medians, minima, and maxima were generated on age, estradiol levels, and BMI z-scores as continuous variables. A Student's t-test was performed on the difference in average BMI z-score by race, considering the t-test corresponding to the result of the F-test for equality of variances. Percentages were also calculated for the proportion of patients within specific age ranges, the proportion of patients with estradiol levels above the highest

LOD, and the proportion of patients with an etiology or related comorbidity. SAS version 9.2 was used for all analyses.

2.4 Hypotheses

Although it appears that black girls reach all pubertal milestones at an earlier average age than white girls, in terms of secondary characteristics, the evidence for breast development is much more abundant. Younger age at menarche is also supported by sufficient evidence, however the number of girls reaching menarche at an age that would be considered clinically precocious (under 9-10 years) would likely be small based on analyses in the general population (47) and therefore should similarly not constitute a substantial proportion of the precocious puberty patient population. Moreover, given the suggestion that age at menarche may be stabilizing while pubertal onset as measured by breast development still appears to be decreasing in age, this trend may be more indicative of breast development occurring in the absence of central activation through the HPG axis (99). Moreover, many of the environmental exposures suspected to influence pubertal timing, as well as those presumed to be more common in black populations, have estrogenic effects that could promote breast development. Therefore, these observations have led to the hypothesis that a much larger proportion of black precocious puberty patients will present with early breast development that is primarily independent of the HPG axis. Namely, this study hypothesizes that premature thelarche will be a particularly more common diagnosis in black girls than white girls.

Since age at presentation for evaluation will be correlated—although imperfectly—with age of pubertal onset, and given the younger onset ages of black girls compared to their white counterparts in the general population, it is further hypothesized that the ages of girls presenting for evaluation will be younger in black girls, even among a sample of precocious puberty cases. Moreover, this difference may be particularly noticeable for breast development, specifically premature thelarche, given the reasons stated above.

Similarly, it is hypothesized that estradiol levels among black patients of comparable ages and with the same diagnosis will be higher than those of their white counterparts. This is based on the assumption that their level of pubertal advancement will be greater at a given age assuming earlier onset and the fact that research conducted in prepubertal girls has previously demonstrated higher estradiol levels in African Americans of a similar age and BMI (100).

Chapter 3: Results

3.1 Diagnoses

Table 1 summarizes the results of the classification of patients into the 4 diagnosis categories according to race. Among the 50 black patients, PT and CPP were equally common, each constituting 28% of all diagnoses, followed closely by PA (26%). The remaining 9 patients (18%) were assigned to the OPP category. Of the 49 white patients, roughly half were diagnosed with PA (49%). CPP was the next most common diagnosis (22.5%). 8 patients (16.3%) were classified in the OPP category, and PT was the least common diagnosis, seen in only 6 white patients (12.2%). The differences in the distribution of diagnoses by race were not significant at the 5% α level but were significant at the 10% level ($p=0.076$).

3.2 Etiologies & Comorbidities

In the group of 11 white patients with CPP, 1 also had optic glioma and neurofibromatosis, 1 had Arnold-Chiari malformation, 1 was found to have a cyst of the pineal gland, and 1 was found to have a cyst of the pars intermedia of the pituitary gland, although a neurologist felt it to be unrelated to her pubertal changes. This developmentally delayed patient also had seizures and apraxia. An additional patient was the result of a premature twin birth and had a history remarkable for a cerebral hemorrhage after birth that was considered a possible cause of her puberty.

One white patient with an initial diagnosis of premature thelarche was later found to have a parahypothalamic hamartoma after entering true puberty. One white patient in the OPP category who did not have true precocious puberty had the genetic condition of Aicardi syndrome that consisted of a lack of formation of the corpus callosum, cysts in the brain, and associated seizures.

Among the black patients with CPP, 1 had a history of hydrocephalus and 1 was found to have a pars intermedia cyst of the pituitary gland as well as ovarian cysts, which are not uncommon in infants (101). In the group of OPP black patients, 1 thalassemic patient's puberty was attributed to an ovarian cyst. It is also noteworthy that another patient who had been born prematurely with complications also had a blood clot on the left side of the brain that eventually dissolved or was absorbed; however, no mention was made of this being responsible for her puberty. An additional black patient diagnosed with PA had a history of stroke after birth and was subsequently developmentally delayed and wheelchair-bound. The doctor does mention her neurological insult as possibly related to her pubertal development.

Cerebral palsy (CP) was also a condition seen in 6 patients. In particular, 3 white patients with PA had CP—1 of whom also had subcortical band heterotopia and seizures; the second had periventricular leukomalacia (PVL) associated with prematurity, spastic diplegia, and a seizure disorder or epilepsy; and the last was described as having CP of the leg. Two black patients with an initial diagnosis of PA had CP, 1 of whom also had microcephaly, seizures and was not ambulatory. One black PT patient had mild CP and also had PVL associated with prematurity. Table 2 presents statistics based on all of these identified neurological impairments in the study population.

Two patients had potential known exposure to exogenous hormones, namely testosterone used by their fathers—although in both cases it was reported that their fathers took precautions to prevent exposure to the family. One was a white patient with PA that was considered to possibly be consistent with exposure. The other patient, however, was a white patient with CPP; she was also known to have seizures with no identifiable cause, and because of a normal MRI, was considered to have idiopathic CPP. No black patients were identified as having exposure to exogenous hormones.

Additionally, a total of 14 patients had acanthosis nigricans (AN), a condition characterized by darkening and thickening of the skin, primarily in skin folds, and associated with obesity as well as insulin resistance (102). Twelve of the 14 patients with AN were black and 2 were white. Thus, 24% of black patients overall and 4.1% of white patients exhibited signs of AN. Of these patients, 5 had an initial diagnosis of CPP, 4 were classified has having OPP—2 of whom were specifically noted to also be insulin resistant—and 3 had initial diagnoses of PA. In one of the black CPP patients, AN was only noted at the last visit. Of the 2 white patients, 1 had CPP and was noted to have AN at a follow-up visit and the other was classified in the OPP category and was noted to also have mild increased insulin.

Other conditions found among the patients without known relation to precocious puberty but which may distinguish them from normal individuals include Apert syndrome—a genetic condition causing early fusion of bones in the skull (103)—found in one white patient with PA; Translocation Down syndrome in a white patient with CPP; thalassemia in a black patient with CPP mentioned above; and Turner syndrome—which results when a female does not have all or part of the second X chromosome (104)—in a

white patient with PA. This patient also later developed Hashimoto's thyroiditis, but this occurred after her premature puberty.

3.3 Patient Characteristics

3.3.1 Body Mass Index

Of the 37 black patients and 45 white patients over 3 years of age for whom BMI was calculated, the distribution of BMI z-scores for both black and white patients was shifted above that of the reference population based on the CDC 2000 growth charts, as the majority of patient z-scores were above the mean of zero for the standardized reference population (Figure 1). Black patients had significantly greater BMI z-scores than their white counterparts, with a mean z-score of 1.43 compared to 0.94 in whites ($p=0.02$). 70.3% of black patients had a BMI qualifying them as either overweight or obese compared to 40% of white patients ($p=0.0062$), with 46% of blacks and 24.4% of whites being classified specifically as obese.

White patients had lower median BMI z-scores than blacks for each diagnosis category except for PT, which contained only 4 whites and 6 blacks in the analysis (Table 3). Median z-scores in white patients for each diagnosis were 0.90 for CPP, 1.78 for OPP, 0.85 for PA, and 0.71 for PT; and in black patients the medians were 1.40 for CPP, 2.24 for OPP, 1.49 for PA, and 0.68 for PT. White patients had a wider range of z-scores in all categories and lower minimum values in all categories except PT, but maximum z-score values were comparable (Figures 2a-d). In both black and white patients, PT had the

lowest median BMI z-scores and OPP had the highest scores; the proportion overweight or obese ranged from 50% for PT to 85.7% for OPP in blacks and from 25% for PT to 57.1% for OPP in whites. The differences in these proportions by diagnosis were not statistically significant for either race ($p=0.50$ & $p= 0.77$ in blacks and whites, respectively; Fisher's exact test).

3.3.2 Income Level

64% of black patients compared to 22.5% of white patients were classified as low income based on enrollment in Medicaid or PeachCare for Kids insurance programs. This difference was statistically significant ($p<0.0001$), with the strength of the association between race and low income given by a risk ratio (RR) of 2.85 (95% confidence interval (CI): 1.63-4.99). This indicates that black precocious puberty patients in the study were 2.85 times more likely to be on Medicaid or PeachCare for Kids than white patients. By diagnosis, the percentage of patients classified as low income ranged from 46.2% in PA patients to 92.9% in PT patients among blacks ($p=0.034$, Fisher's exact test) while low income white patients comprised a minority in each category that ranged from 16.7% to 36.4% and did not differ significantly ($p=0.62$, Fisher's exact test) (Table 4).

3.3.3 Age

The age of patients at initial evaluation for precocious puberty ranged from 0.58 to 9.95 years in black patients overall and from 1.26 to 10.24 years in white patients

overall (Table 5). Age was not normally distributed (Figure 3). The median age for each race was 6.64 and 7.46 in blacks and whites, respectively. In both cases, patients were concentrated between 6 and 9 years; however among blacks, 21 patients (42%) were under 6 years of age and 19 patients (38%) between the ages of 6 and 8 years compared to only 6 patients (12.2%) under 6 years and 30 (61.2%) between the ages of 6 and 8 years in whites. The remaining 10 (20%) black patients were first evaluated at 8 years of age or older, and 13 (26.5%) white patients were also evaluated in this age range.

Within the group of girls diagnosed with CPP, 10 of the 14 black patients were evaluated at 7 or 8 years of age. Of the remaining 4 patients, 1 patient presented for evaluation in each of the years from 4 to 6; and the final patient was 10 months old with breast development and an episode of menses and was also found to have a pars intermedia cyst of the pituitary. Among the white patients with CPP, all 11 presented for evaluation at 6 years or older. In the PA category, all black patients were approximately 5 years of age or greater at evaluation, and two-thirds of white patients were evaluated at 6 or 7 years of age. With the exception of a 2.88 year-old who presented because of excessive body odor without sexual hair but whose biochemical evaluation revealed elevated androgens consistent with PA, all other white girls were at least 5 years old.

Unlike the other 3 categories, no PT cases were 8 years and above at initial evaluation. The majority (71.4%) of black cases presented at 3 years of age or younger, and the remaining 4 of the 14 black PT patients were evaluated at 6 and 7 years. Among the 6 white PT cases, 4 were 6 or 7 years old with the remaining 2 patients being 1-year-olds. In the OPP category, 6 of the 9 black cases were 6 years or older; 1 was a 4-year-old; and the remaining 2 were under 1 year of age. Among the white patients, 7 of the 8

patients categorized as OPP were 6-8 years of age, and the last patient was a 1 year-old who also had Aicardi syndrome. Summary statistics are presented in Table 4 along with plots of the distributions in Figures 4 a-d.

3.4 Estradiol Levels

A total of 73 patients had an estradiol test. Estradiol levels were missing primarily on PA patients (61.5% of black patients and 58.3% of white patients), along with 4 additional patients (1 white patient with CPP, 1 black patient with PT, and 2 black patients in the OPP category). A total of 15 patients had levels that were recorded as being below a LOD, including 5 white patients (OPP: 1 <3 pg/ml, 1 <7 pg/ml; PA: 1 <2 pg/ml; PT: 1 <3 pg/ml, 1 < 7pg/ml) and 10 black patients (CPP: 1 <2 pg/ml; OPP: 2 <2 pg/ml; PA: 2 <7 pg/ml; PT: 2 < 2 pg/ml, 2 <7 pg/ml, 1 <20 pg/ml). Figure 5 presents estradiol levels by race for the entire sample.

Because hormone levels are age-dependent, Table 6 presents statistics for girls only through 9 years of age at the time of testing, in accordance with the ages considered prepubertal in an established reference range.[a] The majority of black patients with CPP had estradiol values of 26 pg/ml or less, apart from 3 patients with results in the 50-60 pg/ml range. 57.1% had levels below 20 pg/ml. In contrast, 33.3% of white CPP patients had levels below 20 pg/ml; however all results were no greater than 35 pg/ml. In the OPP category, levels among black patients were 24 pg/ml or lower, with the exception of a patient whose puberty was the result of an ovarian cyst and who had the highest level of

[a] Pediatric female reference range for Estradiol, ultrasensitive, LC/MS/MS: Pre-pubertal (1-9 years): < or = 16 pg/ml; 10-11 yrs: < or = 65 pg/ml; 12-14 years: < or = 142 pg/ml; 15-17: < or = 283 pg/ml[vi]

69 pg/ml. Estradiol levels in white patients in this category were under 17 pg/ml. For patients with PA, the median estradiol values were similar in the two racial groups. However, levels in black patients did not exceed 14 pg/ml while 3 white patients had results over 20 pg/ml, with one being as high as 40.8 pg/ml in a 7-year-old. The other two patients with elevated levels included a 6-year-old obese girl with a level of 23 pg/ml and a 9-year-old with a level of 21 pg/ml who was in normal puberty at the time and had been followed from age 6.

Among white patients with PT, estradiol levels were measured at 14 pg/ml or lower apart from 1 patient referred with an estradiol result of 34.16 pg/ml. This patient did have a normal result upon a second test performed at her initial visit. Nevertheless, she subsequently went on to progress to true puberty as an 8-year-old by the time of follow-up approximately 8 months later, and an MRI also revealed a parahypothalamic hamartoma. Black patients with PT had estradiol levels under 19 pg/ml except for a result of 48 pg/ml in an otherwise normal 1-year-old girl.

Two additional white patients were older than 9 years of age at the time of their laboratory test and not included in the analysis presented in Table 6 and Figure 6. The first was a patient with CPP who presented at 10.24 years of age and was the oldest patient in the study sample. At this time, she had an estradiol concentration of 111 pg/ml, which was the highest level in the sample and a clear outlier compared to the remaining patients. This level also exceeded that specified in the reference range for her age (10-11 years: ≤65 pg/ml) and fell within the range for girls aged 12-14. The second girl was a patient with PA first evaluated as a 7-year-old and followed for several years as she

progressed normally through puberty before an estradiol test was first administered at an age of 11.46 years with a result of 21.20 pg/ml.

Chapter 4: Discussion

4.1 Diagnoses

The results of this pilot study indicate a substantial difference in the distribution of black and white patients among precocious puberty subtypes. In particular, a much larger proportion of white patients were diagnosed with PA than black patients (49% vs. 28%) and a smaller proportion was diagnosed with PT (12% vs. 28%). There was only a slight difference in the CPP category (22.5% vs. 28%), and the OPP category was nearly identical (16.3% vs. 18%). The differences in these resulting distributions were only significant at the 10% α level, but the failure to achieve statistical significance at the 5% level may be a result of the limited power provided by the small sample size of this study.

Black patients were more evenly distributed among the 3 major categories of precocious puberty (PA, PT, & CPP) compared to white patients; and, as hypothesized, PT constituted a greater proportion of cases in black girls relative to white girls, in whom it contributed the least to cases of precocious puberty. In both races, it appears that precocious puberty in girls is dominated by conditions that are likely not centrally mediated, and the results further suggest that a greater proportion of early puberty in blacks may involve estrogen-dependent changes than solely androgen-dependent changes that predominated among white patients in this study population. For example, apart from PA, the other three categories all contained some level of breast development—even though androgenic changes were also present in OPP and may have been in many CPP cases as well. This implies that nearly three-quarters (74%) of black patients had some

breast development compared to about half (51%) of white patients; and if the CPP group is excluded because the estrogen changes are a definite result of HPG activation, the proportions with breast development that may not truly involve HPG activation are still noticeably different (46% for blacks and 28.5% for whites). Together, these findings may lend support to the potential role of exogenous estrogen-active exposures in promoting early estrogenic pubertal changes that may disproportionately affect African Americans, such as some EDCs that are typically estrogenic in nature, HCHPs containing estrogen, or certain food sources discussed in the introduction.

Although a hypothesized lower proportion of PT in white girls relative to black girls would imply greater proportions of other subtypes in whites relative to blacks, the fact that this was primarily limited to PA and the magnitude of the proportion of white patients with PA was somewhat unexpected. The study published by Kaplowitz (97) on 104 patients also found PA to be the most common diagnosis, constituting 46% of the study population that included both black and white patients; however the inclusion of male patients in the study and the failure to note the race of patients by sex prevents the determination of the distribution of subtypes in girls exclusively. PT was found to be the next most common diagnosis in that study. Kaplowitz does mention that more than half of the PA patients and 74% of the PT patients were black, but blacks also constituted a larger proportion of the overall study sample. Moreover, direct comparisons with this study are further limited by the inclusion of evaluated girls without confirmed puberty in his denominator and the further subdivision of categories—namely, the additional categories of pubic hair of infancy and early breast development considered distinct from PA and PT.

The finding of a much greater proportion of PA in whites in the present analysis does not seem to agree with other assertions that PA is rare and more frequently seen in blacks and could be related to higher rates of insulin resistance in blacks (3, 8, 90), although this statement has been made in reference to the general population as a whole and not a sample limited to precocious puberty cases. Furthermore, as mentioned previously, data from the normal US population demonstrate that both breast and pubic hair development begin earlier in black girls, and given that the pubertal sequence in blacks more so than whites may involve the development of pubic hair preceding breast development (2), one might expect a greater proportion of black patients exhibiting both signs of puberty than white patients, which was not really the case in this analysis.

Another study did find a combination of both pubic hair and breast development to be the most common finding in their population of 168 girls; and although they only presented the racial breakdown for each subtype, calculating the distribution of categories within racial groups from their data demonstrates that both signs were present in 56.0% of blacks and 48.8% of whites followed by pubic hair only in 36.9% of blacks and 40.8% of whites and breast development only in the remaining 7.1% of blacks and 14.4% of whites. Thus, PT represented a greater proportion in whites than blacks, but when combined with both signs the overall proportion is slightly less. However, the age of their population was limited to girls between 6 and 8 years of age, which may explain some of the differences in their distribution compared to the one observed here. Moreover, basing categorization on signs alone does not distinguish between whether pubertal changes are centrally regulated or independent of the central mechanism; since breast development

without pubic hair, for example, may present in CPP (28), which also limits comparability with this study.

4.2 Etiologies & Comorbidities

Of the conditions seen in this study sample, glioma/neurofibromatosis, hydrocephalus, hypothalamic hamartoma, and Arnold-Chiari malformation are recognized causes of CPP, and ovarian cysts are a known cause of PPP (3-5, 9). Although less commonly cited as a cause of precocious puberty, several articles have reported CPP in both girls and boys with pineal cysts (105-109). Likewise, pars intermedia cysts are generally considered benign; however, there are cases in the literature describing precocious puberty in children with Rathke's cleft cysts— a type of cyst of the pars intermedia that forms when portions of the Rathke's pouch, which gives rise to pituitary regions including the pars intermedia, remain after fetal development (110-113).

One patient in the OPP category also presented with Aicardi syndrome that may have had some relation to her pubertal findings according to the endocrinologist, and Aicardi syndrome has been found to be linked to both precocious puberty and pubertal delay (114). Blood clots of the brain, cerebral hemorrhage, and stroke were not specifically found in the literature on precocious puberty; however, ischemia is listed as a cause of CPP (4), and a study on CNS abnormalities in CPP patients in Italy included a patient with intracranial hemorrhage (109). Yet, the patient with a history of stroke and subsequent neurologic damage that was believed to be related to her puberty was

diagnosed with PA while the patient with a neonatal blood clot was in the OPP category, and the endocrinologist made no explicit mention of this in relation to her puberty.

Six patients were described as having CP, which also is not considered a cause of precocious puberty. However, it may have some association with precocious puberty. In an analysis comparing CP children to the normal population, white girls with CP had an earlier average age of pubic hair development than girls without CP, though they started breast development around the same time and ended breast development later on average. Although the average ages for breast development did not differ, girls with CP also had higher percentages of both early and late breast development compared to the distribution in the normal population (115). Five of the six CP patients in this study had PA, and one had PT, which are consistent with these findings. Most CP patients also had additional problems, such as subcortical heterotopia in one PA patient and two patients with PVL (1 with PA and 1 with PT). The Midyett et al. (116) study on girls under 3 years also found 2 cases of PVL, 1 with PT and the other with precocious puberty.

Overall, there appeared to be slightly more CNS conditions among white patients, with a more striking difference in the CPP group, which might imply that CPP is more likely to be idiopathic in black patients. However, this would need verification in a larger-scale study not limited by small numbers. There were an equal number of patients in the OPP, PT and PA group with comorbidities of interest, but because of the distribution of patients across conditions, they constituted a notably larger proportion of PT patients in whites and a larger proportion of PA patients in blacks.

In another study, 12.3% of precocious puberty patients evaluated had what was labeled an endocrine pathology, but race was not considered and the study was limited to

girls 6 to 8 years of age and included AN among the conditions (89), which some do not consider to be a true pathology in and of itself since it usually requires no treatment other than lifestyle changes (97). Examination of the data presented by Midyett et al. (89) excluding the AN patients reveals that 4.8% of those with both breast development and pubic hair had a pathology, as did 7.2% of those with pubic hair alone and 0% of those with breast tissue alone. The study in girls under 3 found that 4% overall had an abnormal MRI, and among the 19 classified as precocious puberty, 5 (26.3%) had an abnormality (116). The Kaplowitz (97) study did not note CNS pathologies except to mention 1 white patient with a hypothalamic lesion requiring intervention. In a selection of European studies, CNS findings among CPP patients in particular ranged from 6% to 19% (14, 98, 109, 117), but differences may exist in the conditions considered. The much higher proportion of anomalies in white CPP patients in the present study in contrast to other findings is nonetheless striking but may be a chance finding among the small sample.

Other abnormalities in the study patients such as Down syndrome, Turner syndrome, Apert syndrome, and thalessemia are not associated with precocious puberty, and in fact, Turner syndrome, and thalassemia are often associated with delayed or failed puberty (118-119), though case studies of CPP have been reported in Turner syndrome (119). However, the patient with Turner syndrome in this study only presented with PA and not true puberty.

85.7% of patients with AN were black, 3 had some indication of insulin resistance, and all were overweight or obese, all of which are findings consistent with the literature on AN reporting a greater prevalence among blacks and darker-toned

individuals and an association with obesity as well as insulin resistance (102). Because AN is associated with insulin resistance, and because of observed associations between PA and insulin resistance, hyperandrogenemia, and, as a consequence, PCOS (8, 31), AN is likewise considered to be associated with PA. In fact, in the study by Kaplowitz (97), 75% of patients with AN were diagnosed with PA. This, however, differs from the current study in which only 21.4% of patients with AN were diagnosed with PA and the rest were split among CPP (42.9%) and OPP (35.7%). Yet, most patients with AN in the other categories also had pubic hair in addition to their other pubertal findings, except for 2 black patients in the CPP category. One of them did have elevated DHEA-S consistent with PA, and her AN was only explicitly noted at the last visit. The other did not have pubic hair on initial exam, though it was noted during follow-up. An additional study found that equal proportions of patients with AN (7 each) had either pubic hair only or both pubic hair and breast development (46.7%), and a remaining patient had breast development only (89).

Despite concern in the literature about exogenous exposure to estrogens from HCHPs particularly in the black population, only 2 white patients had probable exogenous hormone exposure and this was to testosterone. Most reports of testosterone exposure and precocious puberty are on boys (120-123)**,** but testosterone cream has been used to induce pubic hair growth in girls with pituitary insufficiency (124). One of the patients with likely testosterone exposure did present with PA, but the other was diagnosed with CPP—although this was believed to be idiopathic. In a study published in 1995 based on 102 records of patients seen for precocious puberty, 8 were found to have exposure to estrogenic HCHPs, all of whom were black (76) . It is possible that this

exposure could be becoming decreasingly common, or that such forms of exposure to exogenous hormones do indeed exist but were not reported if patients are unaware of such exposures. In fact, the above study by Zimmerman and Francis (76) found that none of the parents of the eight children using HCHPs were aware that their children's hair products contained hormones, but it was only discovered when the products were brought for examination.

4.3 Body Mass Index

Analysis of BMI revealed that both black and white patients were heavier than the average age-matched girl based on the CDC 2000 growth charts of the US. In addition, blacks were significantly heavier than whites on average. Because these results were found in a population consisting of only precocious puberty cases, this finding underscores the fact that obesity does not solely explain the occurrence of precocious puberty and racial differences in pubertal timing since white girls continued to weigh less than black girls in this sample. Because differences in the distribution of diagnoses could affect results if a diagnosis was overrepresented by one race and if BMI also has stronger associations with specific diagnoses, analysis of BMI was also stratified by diagnosis, which demonstrated that the proportion overweight or obese did not differ significantly by diagnosis and that blacks continued to have a greater frequency of overweight or obese in all categories. Similarly, in analyses of national survey data controlling for weight, racial differences in pubertal development persisted (22, 33, 47). However, in

precocious puberty patients 7-8 years of age, Midyett et al. (2003) do not report a significant difference in the BMI of black and white girls with the same pubertal signs.

The mechanisms through which obesity leads to earlier pubertal timing have not been clearly delineated; and the process may be a result of increased weight priming the HPG axis and causing earlier puberty through the central mechanism; the production of estrogen by adipose tissue promoting estrogenic pubertal changes outside of the central mechanism; or a combination of the two (99). In this study population, patients with a PT diagnosis appeared to have the lowest BMI on average, which would not lend support to the mechanism purporting estrogen production from fat tissue. However, as mentioned, the results are based on small numbers. Nevertheless, it does suggest that true breast tissue was detected as opposed to fat tissue mistaken for breast tissue. Patients in the OPP category, on the other hand, had the highest BMI, which might provide evidence for the adipose-derived estrogen mechanism if these patients truly do not have centrally-regulated precocious puberty.

One study comparing girls with true CPP and those with unsustained or slowly progressive precocious puberty found that the mean BMI was higher among true CPP patients (11), which differs from the current study results. In the Midyett et al. (2003) study, girls with both breast and pubic hair development had significantly higher BMIs than those with only breast development and significantly higher weight z-scores and proportion obese than girls with either breast development or pubic hair; however, they assume that these girls represent true precocious puberty. The Kaplowitz (97) study found that girls with PT and girls with pubic hair of infancy were around their ideal body

weight while other diagnosis categories were heavier than their ideal body weight, though the study had limited power to detect significance except in PA.

4.4 Income Level

Similar to BMI, the fact that among only cases of precocious puberty, black girls were more likely to come from low income families than white girls overall as well as within each subtype implies that income does not explain racial differences in pubertal onset. As briefly mentioned in the introduction, some studies have demonstrated an association between socioeconomic variables and puberty and have suggested that the link may operate through body weight since individuals of lower SES are more likely to be obese in the US (58). Other mechanisms suggested include differential environmental exposures by SES and/or factors affecting stress response, including social support (61, 125). For example, one study found that lower SES at age 7 was associated with an earlier age at menarche, and this relationship held after adjusting for race and numerous other factors including maternal age at menarche (58). Moreover, the addition of BMI into the model did not eliminate the significance of SES; and in another study on father absence, higher BMI, low income, and African-American race were independent predictors of earlier onset of breast development (61).

Thus, despite some evidence of a link between SES and puberty that is not explained by other factors, the results of this investigation indicate that at least family income level as measured by type of medical insurance does not account for racial differences in pubertal onset because of a persisting racial differential in income level

among these cases of precocious puberty. Similarly, the fact that race was still a significant variable for breast development in the Deardorff et al. (61) study when low income was accounted for also suggests that income does not explain racial differences in pubertal onset, as does the analysis of NHANES III data that found black girls still had significantly greater odds of attaining all pubertal milestones after SES was adjusted for (33).

Interestingly, the results of this analysis revealed a significant difference in the proportion of low income patients by subtype among black patients but not among white patients. This may be a chance finding or an indication of different effects of SES factors on puberty by race. One study did find an interaction between income and race in terms of age at menarche, with blacks having a significantly earlier age at menarche with higher family income while whites had a significantly later age at menarche at the highest income category (125). And in a different study, higher income in combination with father absence was associated with earlier pubic hair growth in only black girls, although income alone did not predict pubic hair onset (61).

Specifically, in the present analysis, the proportion of black patients on Medicaid or PeachCare for kids was roughly equal to the proportion that was not low income in the CPP and PA groups—exactly half were low income in CPP and 6 out of 13 were low income in PA—and a modest majority of two-thirds of patients in the OPP category were low income, but this group had the smallest sample size of 9. However, the overwhelming majority—all but 1, or 93%—of PT patients were low income. The striking difference in this subtype compared to the others might suggest that low income has some role in the timing of breast development, as was found in the Deardorff et al.

(61) study. Considering that BMI was the lowest in the PT group—although a larger number were excluded from BMI analysis because many were under 3 years and assumed to have a recumbent height reading—and the fact that previous studies have shown an association with SES factors independent of BMI, this could imply that income hypothetically operates through another mechanism that promotes estrogenic effects independent of the HPG axis, such as increasing the likelihood of exposure to certain estrogenic compounds among blacks in particular. As mentioned in the introduction, some EDCs have been found in greater frequency among African Americans already, but analysis of differences among income levels needs to be explored.

4.5 Age

Although the persistence of higher BMI and low income levels among black precocious puberty patients might suggest that these factors are not the main reason for racial differences in early pubertal timing in the US, it does not eliminate the possibility that they may have an effect on the degree of precocity—although the findings in the normal population mentioned previously of younger ages of pubertal development in blacks while controlling for BMI and SES do not seem to support this. Nonetheless, another aim of the present study was to determine whether even among early developers, a difference in age exists. Overall, the results of patient age at initial evaluation seem to suggest that black patients may be appearing at younger ages than white patients based both on average measures and the proportion of patients in different age groups. For example, the fact that most of the patients who were under 3 years of age and were thus

excluded from BMI calculations were black (11 of the 15) is indicative of a greater proportion of black patients at very young ages. Moreover, there were 5 black patients presenting at less than 1 year old but no white patients under 1 year of age, and the only 4 white patients who were younger than 3 years appeared as outliers in the overall analysis. Again, this may be influenced by differences in the distribution of patients in subtypes by race if subtypes differ in the ages associated with them, but analysis by diagnosis also seems to confirm younger ages in black patients, though small sample sizes limit the reliability of these findings. For instance, the average ages for black girls were younger for each category except PA which appeared to be similar between the two but still had a slightly younger distribution.

The youngest patients were found in the PT subtype, which is in keeping with other observations and the nature of the condition (5, 8-9). For example, in a study on girls under 3 presenting for early puberty, 80% had PT only (116). However, based on the present analysis, it also appears that PT is even more dominated by young children among black girls in particular compared to white patients, though again, this observation may be affected by small numbers among whites. In this study, 71% of black patients were 3 years or younger compared to 33% of the 6 white patients with PT; and 8 of the 11 black patients under 3 years overall were diagnosed with PT compared to 2 of the 4 white patients under 3 years of age overall. Examining the data presented in the Kaplowitz (97) study similarly reveals that the majority (82.4%) of black patients with breast development were under 3 years of age compared to slightly less than half (45.5%) in white patients.

Moreover, in both black and white patients in this analysis, there was a gap in ages at which PT patients presented for evaluation; none appeared between 2 and 5 years in whites or from 4 through 5 years in blacks. In fact, Kaplowitz separated patients over 3 years from the PT group as another category in his analysis, and a Danish study discusses how PT at 6 years and up may differ from PT in infants (98).

An explanation for the disproportionately larger fraction of black PT patients being infants and toddlers is not clear. BMI of patients older than 3 was not any greater and was actually less in black patients, decreasing the possibility of this as an explanation—although the sample size on which the estimates were based was very small. Since BMI is not a reliable measure for infants (126), other measures would need to be compared to see if size may have any role in the difference between blacks and whites, such as weight-for-length. Some speculative reasons might include differences in the sensitivity of breast tissue to estrogen, including remnants from *in utero* exposure through the mother, and to the "mini-puberty" period of HPG activation in infancy; or another differential environmental exposure early in life to which breast tissue responsive. For example, this finding may be in line with concern over phytoestrogens in soy-based infant formula and the fact that the Women, Infants, & Children (WIC) program covers formula for low income recipients and frequently suggests soy formula to African Americans due to higher rates of lactose intolerance and dairy allergies in this population (7). The use of low-income medical insurance programs was nearly universal in the PT group compared to the rate in other subtypes; and differences in Medicaid eligibility for children based on age do not seem to explain the larger proportion of black PT patients using this service, as the income limits decrease with child's age for Right

From the Start Medicaid for children.[iv] Thus, these results may also lend support to the hypothesis about soy formula. However, as noted in the introduction, studies linking phytoestrogen levels to pubertal development and precocious puberty have not been consistent.

Both black and white patients with CPP had the oldest median age at initial evaluation as well as the oldest maximum age compared to other diagnosis categories, and a similar result was demonstrated by Kaplowitz who found this group had the oldest mean (97). This seems to suggest that much of sexual development seen in precocious puberty patients occurring at a very early age is not true centrally regulated puberty.

The results also seem to indicate that a large proportion of patients present for evaluation at 6-8 years, the ages under question for revision of the precocious puberty guideline, and seemingly more so in white patients than black patients according to this study—which may bolster the arguments of those supporting such a revision if it is common at this age.

4.6 Estradiol Levels

In both races, CPP patients had generally higher estradiol levels and a greater proportion at pubertal levels, which would be expected given that true puberty is occurring. The average levels for CPP patients did not differ greatly between the two races, although there were some noticeable differences in their distributions. While a greater proportion of white patients overall had levels in the pubertal range, more black patients had high concentrations. In the OPP category, white patients did not have

estradiol levels that were clearly in the pubertal range, and all but 2 black patients—including 1 whose result was driven by an ovarian cyst—had levels that were prepubertal. Estradiol was not commonly measured in patients with PA, which is also expected given the fact that it is only dependent on androgens; but for those in whom it was measured, none of the black patients had levels that might be considered truly pubertal, although a few white patients had elevated levels, one of whom was then in normal puberty at the time of the test. Kaplowitz (8) does acknowledge that laboratories may produce estradiol results that can sometimes be erroneously elevated in patients with PA —and likewise for PT—which can be misleading. Moreover, estradiol is known to increase several months ahead of breast development (6), though the two PA patients with pubertal levels but without breast development did not return for follow-up to assess this.

Overall, there does not appear to be a suggestive pattern of racial differences in estradiol levels. While a greater proportion of white patients had levels above 20 pg/ml in several categories, black patients had higher maximum values in all categories that were not limited to androgen signs only, namely PT, CPP, and OPP. When the patient with the highest estradiol value and an underlying cause (ovarian cyst) is not considered, the maxima remain higher in blacks for these categories. Though not presented here, if the 16 pg/ml cutoff for prepubertal levels in the reference range is considered as opposed to the 20 pg/ml cutoff based on the highest LOD in the sample, both the median estradiol level and percentage of patients at pubertal levels are then in agreement indicating that black patients have higher concentrations of estradiol in the PT and OPP categories—because the proportion rises to 38.5% for PT in blacks but is unchanged in whites—while white patients with CPP and PA still have higher levels based on these two measures. Yet, from

this analysis, it may be difficult to discern meaningful differences in estradiol levels by race, but there may perhaps be some indication of differences in the distribution and the extent of elevation of estradiol by race. However, the small number of observations may limit the reliability of these findings, and the width of the age range considered (0-9 years) may have also affected the ability to observe differences if the underlying age distributions also differ and if there is a dose-response relationship with age.

Although race was not assessed, another study found mean estradiol levels of 8.8 pg/ml, 7.0 pg/ml, and 4.6 pg/mol in girls with PT, PA, and precocious puberty, respectively; but this study was limited to girls under 3 years of age (116). An analysis of 20 patients with unsustained or slowly progressive precocious puberty found that none of the patients' initial results were above the LOD of 20 pg/ml (11), which is at least comparable to this study's finding in white OPP patients.

In terms of environmental exposures in relation to estradiol, it is possible that some substances with similarities to estrogen, such as many EDCs, may lead to the down-regulation of estrogen internally, which could be reflected in lower estradiol levels. In a study on women undergoing in vitro fertilization (IVF), higher urinary BPA levels were associated with lower peak estradiol concentrations (127). Moreover, granulosa cell studies have demonstrated inhibition of estradiol production in the presence of both BPA and a phthalate ester (128-129). Thus, depending on how influential this potential down-regulation may be on internal processes and in children in particular, and depending on the level of exposure, it is possible that estradiol levels may not be exceptionally elevated in girls presenting with early puberty if such exposures are in fact playing a role in pubertal development. In fact, in reference to a Danish study on pubertal timing that

found estradiol levels in girls 8 through 10 years of age to be significantly less than in girls studied 15 years prior although breast development was commencing earlier, it was suggested that breast growth in the new cohort may be promoted more by estrogenic effects operating independently of the HPG axis (24). In this analysis, estradiol levels were lower in the categories that were not centrally regulated compared to CPP, though this would be expected regardless of environmental exposures. Nonetheless, the fact estrogen effects such as breast development were still present in other categories even if estradiol levels may not have always been elevated may support this suggestion.

4.7 Strengths and Limitations

One limitation of this study is that the analysis of patients' ages was based on age at initial evaluation as opposed to age at onset; and although the two will be correlated, the age at evaluation would be older, with substantial differences possible in some cases. For example, a number of patients were already 8 years old when first evaluated and several also came for evaluation in their 9^{th} year, as well as 1 patient who was 10 years old. Many of these patients were often presenting after their puberty had advanced to a point that it was a greater concern to the family; for instance, some patients came for evaluation after having started menses, including 2 9-year-olds and the 10-year-old. Others may have been referred prior to their 8^{th} birthday but not had an appointment scheduled until afterwards. There are some inherent difficulties in analyzing onset age, given the vagueness and often inconsistencies in the ages reported in the patient history; and Kaplowitz cites this imprecision in discussing his decision to analyze only age at

patient visit and not onset (8). Moreover, onset information is not as widely available in the charts, although it was fairly common in this data. Despite this limitation, the findings were still suggestive of some age differences by race, but an attempt can be made to approximate onset age in future analyses and determine whether results differ.

Another limitation is the fact that evaluation by a pediatric endocrinologist—at ECC and elsewhere—is based on referral, and referral patterns may be a potential source of bias if referral is not done equally for all patients who present with early signs of puberty. In terms of age, if primary care physicians are aware of earlier maturation in blacks than whites, they could possibly have less concern and delay referral in black patients, increasing the time between onset and referral. Likewise, a similar practice could increase the time between referral and initial visit. Either could result in a seemingly older age in black patients, meaning that in actuality there could be a bigger age difference between blacks and whites. Alternatively, awareness of the revised guidelines in particular may make doctors less likely to refer older precocious puberty patients from 6 years in blacks and 7 in whites. This could make the age distribution appear younger, with a greater effect in blacks. This possibility, however, does not seem to explain the much larger proportion of black infants with PT since if PT was occurring at a similar rate in white infants and constituted the majority of actual white PT patients, they would presumably be equally referred and thus comprise a larger proportion of the study sample.

Time lag for initial evaluation may also differ by diagnosis subtype and could differentially bias the ages of specific diagnosis categories. In particular, appointments may be more urgently scheduled for signs indicative of CPP. Thus, less priority could be

given to patients exhibiting signs of PA, which is not likely to be confused with other subtypes, increasing the age of patients in this group. Moreover, these potential practices could possibly affect the distribution of subtypes themselves. Longer delay can allow for further progression or new signs to appear, leading perhaps to more patients in the CPP or OPP categories. However, proportions in these categories did not differ much by race.

Additionally, certain subtypes may be more or less likely to be referred at all. For instance, awareness that early pubic hair development may be more common in black girls but may not be perceived to be common in white girls may increase referral of white PA patients and decrease referral of black PA patients; and this subtype was found to have large differences. Yet, knowledge of earlier breast development in blacks would also seem to be just as common, and the likelihood that PT in whites would not be referred in favor of PA if it were more frequent seems low.

There is also the possibility of the referral process affecting the proportion of patients presenting with pre-existing medical conditions that may relate to precocious puberty, though it would not affect such conditions identified on examination, such as brain lesions revealed upon MRI. This may increase the proportion of patients found to have abnormalities if there is greater concern about their puberty; however, other US studies in referral populations would experience the same bias, so it is not likely to account for the difference seen in the frequency of CPP pathologies in this study compared to other studies, although overall frequencies as opposed to race-based frequencies cannot be estimated from this analysis for comparison as the racial distribution of patients was fixed. Moreover, comparison to the more widely available European-based studies on CNS abnormalities is also limited by the fact that differences

in the health care system might mean the results are not subject to the same referral processes.

Patient BMI could also potentially be affected by referral if knowledge of a link between obesity and pubertal onset decreases concern among overweight patients who do have pubertal signs, thus making the BMI distribution appear somewhat lower than in actuality; but this would seem to equally affect both races and thus not result in any bias in the differences in BMI observed between the two. In terms of income levels, there does not seem to be any reason for differential referral based on medical insurance.

The possible referral patterns discussed based on awareness of general trends in pubertal development by race could extend beyond doctors to the families' concern about their children that would motivate them to seek medical attention in the first place, which could similarly affect age, diagnosis distributions, and the percentage with other pre-existing morbidities. This would lessen how reflective of the actual population any study based on medical records instead of random sampling can be. Nevertheless, while the study may not be valid for the general population, it is representative of a referral patient population.

The basis of BMI calculations on measurements from the initial visit at ECC also may not reflect BMI at or before pubertal onset—which may be the time period most relevant to understanding precocious puberty—and it may also represent various stages of patients' pubertal development since patients could present for evaluation at different times in relation to their onset. Although earlier growth measures were available for some patients, historical growth data was not uniformly available in patient charts, and differences in measurement methods used at different medical offices and in their level of

accuracy—particularly for height measurements—would have introduce some inconsistencies into the data. Therefore, the earliest growth measurements at ECC were used systematically for all patients; and an association between this BMI and previous patient BMI is still likely to exist, given that childhood BMI shows some consistency over time, particularly among obese patients according to a study by Wright et al. (130).

A small number of patients were evaluated by endocrinologists prior to presenting at ECC and may have been previously placed on treatment, which would decrease the heightened linear growth velocity associated with CPP as discussed in the introduction; but only 3 of the 7 patients evaluated previously were treated with GnRHa therapy, and the most likely effect would be to maintain a BMI closer to this earlier time of initial evaluation, which would be more desired for this analysis.

Additionally, the use of enrollment in Medicaid or PeachCare for Kids in this study is only an approximation of income level—which is itself only one facet of SES—but its basis on income limits makes it a fairly reliable measure of income. Although the PeachCare for Kids program represents higher income levels than Medicaid, small numbers in this category (only 6 patients were in PeachCare for Kids and in an additional 8 patients, it was unclear in which of the two programs they were enrolled) as well as a small sample size overall made a more graded analysis of income infeasible. The low income variable was also based on enrollment in these two programs at any time during their evaluation, and there were 7 patients for whom it was noted that there was evidence of an additional source of medical insurance either concurrent with, prior to, or following enrollment in these low income programs. These patients may thus represent a different income history with possible periods of higher income than those solely in these

programs. However, in terms of effects on results, the fact that 5 of these 7 patients were white would suggest that the association between low income and race may be even stronger in actuality.

Furthermore, there may be additional reasons for Medicaid enrollment, particularly the Medicaid program for medically needy individuals who may not meet the income limits otherwise but whose debt from medical expenses can be applied to compensate for the difference between their income and the income limits.[iv] The precise reasons for patients' Medicaid enrollment cannot be discerned from the information in the medical chart, yet the ability to enroll for medical reasons is still a reflection of financial need. Moreover, slightly more white patients had disabilities than black patients; therefore, accounting for this possibility would again likely strengthen racial differences in income level.

A limitation in the analysis of estradiol is the treatment of undetectable levels, which were approximated based on the LOD divided by the square root of 2. Thus, the validity of summary measures such as means and, to some extent, medians is questionable. The use of a replacement value was applicable to about one-fifth of the data, which, although not an excessive rate, may be more influential when further subdivided into smaller categories. More complex techniques of estimating undetected observations may offer improvements over replacement methods (131), however, the method used in this study is considered reliable and is commonly used despite its limitations (132). Moreover, although descriptive statistics may have bias, probabilities based on categorizing estradiol levels at the LODs are valid.

The estradiol test results on which this study's analysis was based were taken either before the initial visit by a referring physician (n=23), or at the initial evaluation for precocious puberty (n=40). Four were taken after the initial visit but within days or weeks of it; but the only available estradiol test for 6 patients occurred during follow-up months and, in some cases, years after the initial visit. Ideally, comparing only those tests at the initial visit or before would be preferable, though it would further reduce the sample size. Moreover, apart from 1 of the patients described separately in the results, the remainder of the 6 patients who had follow-up test results were still tested at ages within the prepubertal reference range, and none of them had estradiol levels that exceeded those of patients with the same diagnoses who had earlier tests.

Furthermore, analysis of individual laboratory test measures is always limited by the fact that it represents a level at only one point in time which may in actuality be subject to variation and fluctuation over time.

Because the study was limited to patients who had evidence of precocious puberty that was confirmed on examination, misclassification of individuals with actual prepubertal status—such as fat or body hair mistaken for breast tissue or pubic hair, respectively—is less likely. An attempt was made to systematically assign patients to diagnosis categories based on the endocrinologists' diagnosis and the patients' profile, but the potential for some instances of misclassification—though likely rare—may still exist given the nature of precocious puberty itself, namely the fact that subtypes of PT and true CPP are not completely distinct but constitute a spectrum (12). Thus, breast development in older girls can particularly pose difficulties in distinguishing between the two subtypes (133). As mentioned previously, the Kaplowitz study distinguished a

category labeled as “early breast development not defined” which the author describes could potentially be considered “later-onset premature thelarche, slowly or non-progressive precocious puberty, or very early true precocious puberty” (97), which again reflects the continuum that exists.

Furthermore, slowly or non-progressive precocious puberty is sometimes described as one variant of CPP in which pubertal changes remain stable or recede and the HPG axis does not appear to be activated (12). Palmert et al. (11) conducted a study on patients who had both androgen and estrogen signs but whose response to GnRH stimulation and clinical course did not quite demonstrate true CPP and therefore concluded that unsustained or slowly progressive puberty was a separate entity. Many patients in the OPP category in this study may bear similarities to those in the Palmert et al. study; but ultimately, if follow-up does not occur, a GnRH stimulation test is the best way to definitively distinguish CPP, which was not frequently performed in this patient population (4 patients had a GnRH stimulation test, and 1 had an ACTH stimulation test to assess adrenal pathology in PA).

It should also be noted that two patients in the PA category did not present with sexual hair but had biochemical profiles consistent with PA. It has been demonstrated that biochemical evidence of adrenarche commences 1 to 2 years before pubarche (134).

This study excluded patients of races other than black/African American and white/Caucasian as well as those identified as biracial and Hispanic/Latino. Thus, it approximates a non-Hispanic white and non-Hispanic black population. However, there is the small possibility of some misclassification, especially considering that race was not self-reported but assigned either by the doctor in his or her assessment or during the chart

review based on an included photograph. In particular, there may be difficulties in distinguishing some patients who are biracial from those who are considered black/African American, for example, and the distinction may not be made with perfect accuracy by a doctor or other observer in the absence of inquiry into patients' background. However, when race was judged by the author based on photographs provided in the charts, those for whom there was any uncertainty were not included.

Additionally, there is also the possibility for some patients who may be considered Hispanic black or Hispanic white to have been described in their chart by the physician solely as black or white and thus included in the sample, making it not exclusive to non-Hispanic ethnicity. In any case, these possibilities of race misclassification are not likely to have comprised a large portion of patients if they occurred at all, and their relevance to the study would depend on whether biological or socio-cultural considerations of race are pertinent—in the former case, it might be assumed to obscure observed racial differences while in the latter, this would be less of a concern. Ultimately, the concept of race is always very complex, with any given racial group being a description of physical features that encompass a wide variety of genetic traits and mixtures and with each having social and cultural meaning as well.

Because the aim of this study was to select equal numbers of patients of each race in order to ensure that each group's sample size was sufficient for analysis, the study does not reflect the actual racial composition of the precocious puberty patient population, so results on the total study sample as a whole would not be valid and were thus not generated. From the list of charts provided, it appears that more patients evaluated were black since their target sample size was reached earlier and the remaining 17 otherwise

eligible black patients from the list had to be excluded while an additional white patient was still needed for the sample after all patients on the initial list had been reviewed.

However, race was not available in 163 of the 360 (45.3%) total charts seen, including 38 patients (10.6%) who only had photographs. Because race was not self-reported, self-selective omission of race information would not be a potential source of bias. Yet, the failure to note race in some patients' charts may depend on which doctor is seeing the patient, and there could possibly be a racial differential in the likelihood of recording race—for instance, if doctors are more likely to explicitly note non-white race and omit race in white patients if it is viewed as the "default". Of the 38 total charts with only pictures and no other race information, 25 (65.8%) did belong to white patients, and 17 of the included 49 white patients' race data (34.7%) was based on a photograph only compared to 3 of the 50 included black patients (6%). Nonetheless, given that roughly equal numbers of each race were still obtained and if there is no relation between missing race data and the measures analyzed, this would not constitute a source of bias in the results. In the future, the ability to make contact with patients to obtain race where it is missing could be explored and would allow for a more complete sample.

Other reasons for exclusion from the study included patients who were initially evaluated outside of the study date range and those whose evaluation did not result in a diagnosis of precocious puberty being confirmed because of an absence of pubertal signs, a judgment of normal age-appropriate puberty, or an unrelated diagnosis included in the list. Thus, none of these additional exclusions should lead to biased results.

The sample was selected based on consecutive examination of charts according to the terminal two digits of the MRN, and observations during the chart review process as

well as information from the staff member responsible for providing charts seem to suggest that MRNs are assigned at random and that the terminal two digits of the MRN are unrelated to patient characteristics.[b] Since all eligible white patients on the initial list were included in the study, the method of assigning MRNs would only be relevant to black patients for whom remaining eligible patients were excluded once the target sample size was reached.

Because of the limited sample size and, therefore, power of this pilot study as well as the potential for problems associated with multiple comparisons, additional statistical tests were not carried out; but further pertinent analyses could include testing the significance of differences between blacks and whites for each diagnosis category in terms of variables such as BMI and income level. Although the summary results present this data and illustrate some differences, the significance of these differences was not verified in each diagnosis category and was only tested on the racial groups overall. Moreover, statistical tests on the age distribution, estradiol levels, and the proportion of patients with comorbidities were also not carried out.

A larger scale study is needed to replicate the findings of this pilot study and provide sufficient power to detect statistical differences in the analysis results. Larger studies can also enable more specific analyses to be conducted, such as stratification by factors including BMI and SES in comparisons of the distribution of diagnosis categories.

Despite the limitations, there are several strengths to this study as well. Its reliance on diagnosis subtypes, which encompass both physical signs and biochemical

[b] MRNs for patients seen during the study time period were generated by the Health Information Services department according to preprinted record covers with assigned numbers that came in cases, and these were assigned to patients by the front desk staff (Natasha Quinones, Emory Children's Center, personal communication, 2011).

evidence from laboratory results that inform the diagnosis as opposed to relying on physical signs alone—as presented in the study by Midyett et al. (89) or in studies based on national surveys of the U.S. population—is one major benefit of this study. In addition, it tests for differences in the distribution of subtypes by race, which has not been done in other studies of US precocious puberty patients; and other patient characteristics—such as age, BMI, estradiol levels, and neurologic problems—are also compared by race, which has not been found in the literature specifically on precocious puberty patients as a whole, let alone within diagnosis categories, apart from the analysis of growth parameters by race in Midyett et al. (89). Nor have the few other patient studies that do exist included an SES measure in their analysis. Furthermore, the emphasis on consistent and precise measures, such as unrounded BMI z-scores and age values for greater precision, and on analysis of distributional differences as opposed to depending on only measures of central tendency that are susceptible to the effects of skewness, extreme observations, and small numbers are also strengths of the study that reveal more detailed information about the nature of the parameters in question.

4.8 Future Directions

Additional analyses should also examine racial differences in other features of precocious puberty, such as tanner stage, bone age, and height velocity. In particular, while the present analysis focused on initial diagnoses, analysis of progression, accounting for differences in follow-up time, and of the course and pace of patients' pubertal development in relation to other factors will also be useful to determine whether

advancement or changes occur. Considering that a tenth of PT patients go on to develop CPP (4), this may be important. Furthermore, several patients had evaluations beyond the approved study period, and others will continue to be evaluated; so extending the study time frame would also provide the opportunity to continue to follow patients for even longer time periods as their evaluation continues. Moreover, additional laboratory tests—including gonadotropins, other sex steroids, and androgens—should be analyzed by race and diagnosis in order to assess differences in other biochemical measures as well.

Other parameters associated with pubertal development can likewise be analyzed in this data, such as maternal age at menarche and relevant family medical history, birth weight or gestational age, and household composition—namely, the presence or absence of a father—to see if racial differences and differences by diagnosis exist. Additionally, medications that affect growth, such as steroids prescribed for asthma (135), may need to be considered. Furthermore, the pregnancy history of patients' mothers could also be examined both for complications as well as exposures including medications or substance use if *in utero* exposures and conditions may be of interest.

Sensitivity analyses re-analyzing some variables such as BMI and age to exclude patients with disabilities or abnormalities can determine whether alterations in the results occur. An examination of the patients with the most extreme BMI and growth measures did not reveal any abnormalities, and the few patients with significant health concerns and extreme values for age were noted in the results; nonetheless, formal sensitivity analyses may be useful in the future.

Similarly, some have suggested that estradiol levels may assist in distinguishing idiopathic from pathological CPP resulting from CNS abnormalities (14, 116); however,

estradiol levels did not differ significantly in certain studies (116-117), and it is further recognized that the general estradiol assays that are not sufficiently sensitive limit their utility in distinguishing between precocious puberty subtypes (9, 16-17). In this study, apart from the patient with an ovarian cyst, the remaining high estradiol levels above 50 pg/ml were found in normal CPP patients without CNS abnormalities. However, a systematic analysis of lab results according to the nature of patients' conditions may also be useful to determine if differences exist.

Moreover, analyzing some parameters in new ways can also be insightful. As mentioned previously, analysis of onset age can be attempted as a more useful measure of age for comparison by race. Similarly, area-level income can be assessed using patient ZIP code abstracted from the charts in order to examine patients' socio-economic environment that may reveal additional information beyond individual-level measures and can provide a numeric value for income as well.

Future analysis should also focus on assessing differences in the management of precocious puberty patients. As mentioned above, a difference may exist in the time between the referral and initial visit that may vary by diagnosis category and possibly by race, and this time interval may be able to be discerned from charts with adequate outside records on the visit leading to referral. Likewise, analysis of the occurrence of a follow-up visit, total duration of follow-up, and interval between follow-ups by diagnosis, level of pubertal advancement, and race could provide some insight into the management of patients. Furthermore, another indication of patient management could include assessing which tests are ordered for patients based on diagnosis as well as race. Similarly, the assessment of treatment information including the reasons for treatment, treatment type,

age at treatment, and treatment duration can be examined in relation to race and patient comorbidities, given the consideration of developmental delay and patient's ability to manage puberty in making treatment decisions mentioned in the introduction. In addition, analysis of doctors' impressions in relation to the clinical and laboratory characteristics of patients can also be assessed to see if racial differences exist.

Future studies may also consider the inclusion of other racial groups for additional comparisons, including biracial patients, which could be useful in assessing the existence of gradients in results.

Finally, given the environmental health implications that this study sought to understand and that the results may have suggested, future research should include the measurement of exposures of interest in this population as well as a comparison group, such as serum testing for EDCs, measurements of EDCs and hormone content in PCPs as a possible source of exposure, and interviews with caregivers on potential sources of hormonally-active substances such as food sources and other suspected routes of exposure.

4.9 Conclusions

In summary, the results of this pilot study demonstrate that racial differences may indeed exist between black and white girls with precocious puberty. In particular, there is a suggestion of a difference in the distribution of subtypes of precocious puberty, with PA differing most between the two races, followed by PT, but with minor differences in CPP and OPP. The results further suggest that PA is the predominant subtype in white

patients presenting for evaluation; and while early breast development may be more common in black girls with precocious puberty than white girls, true precocious puberty does not appear to be responsible for this. Racial differences may also exist in the age of patients and possibly in the frequency of pathologies and other comorbidities, though the meaningfulness of these results may be limited by the small study sample.

The implications of these findings for environmental health research are that there is a very likely possibility that hormonally-active environmental exposures may be involved in racial differences in precocious puberty in particular and, by extension, in racial variations in the trend towards earlier puberty in girls in general. The fact that other potentially explanatory factors, such as weight and low income, still differ significantly by race despite a shared precocious puberty diagnosis lends more credence to the need to explore non-traditional factors such as environmental exposures that may be differential according to race, as well as the reasons for these potential differences in exposure levels. In the case of African-Americans, there may be increased exposure to substances that are estrogenic in nature, such as higher exposure to certain EDCs or hormone- or placenta-containing hair care products. The striking number of black infants with PT, coupled with the disproportionately high rate of low income in this group may emphasize the need to consider early life exposures, such as infant feeding practices or *in utero* exposures.

Some may view these results—particularly the high proportion of patients presenting over 6 years of age—as indicative of the appropriateness of the revised guidelines for precocious puberty that recommend a lower cutoff of 6 years for black girls and 7 years for white girls. However, one must also be cautious of the possible ramifications this may have, namely that it could lead to an acceptance of earlier pubertal

development without an attempt to understand the causes of this phenomenon. Similarly, it may lead people to conclude that racial differences in pubertal development are inherent without regard for environmental factors that may play a role in promoting these differences. As Slyper states in relation to the concern over the possibility of overlooking patients with pathological causes as a result of the proposed guidelines, “the fact that ‘normality’ may have changed does not negate the possibility that the physiological processes leading to these changes are neither normal nor benign” (31). This argument can be extended further to include the fact that the environmental factors behind earlier pubertal onset may likewise be disadvantageous. Furthermore, it has also been argued that less emphasis should be placed on the use of age criteria as the basis of precocious puberty diagnoses and more focus should instead be given to the nature and rate of progression of the pubertal changes (9).

This study also contributes to the expanding body of work on race and health. Emerging areas in this field of research have also focused more attention on the role of racial differences in the life experience itself related to living in a society in which race is important and the stresses and subsequent physiologic responses that may be associated with this. For example, the notion of premature “aging” among African Americans has been suggested as an explanation for some racial health disparities (136). Such hypotheses have not been applied to racial differences in pubertal development—although Deardorff et al. (61) do suggest the possibility of stressors such as discrimination or poorer self-image that may operate at greater levels in higher-income black girls in discussing the observed interaction in which high income with father absence in African Americans predicted earlier pubic hair, given the link between the

stress hormone cortisol and other adrenal hormones. Thus, these notions may also be a new area for exploration.

Regardless, it has been recognized that epidemiologic studies on race and health should move beyond solely documenting racial disparities in health outcomes and move towards attempting to understand and account for them (137-139). By using descriptive findings on racial differences in precocious puberty in girls in order to suggest plausible pathways and exposures that can inform future associative and etiologic research on the role of the environment in the racial divergence in pubertal timing, this study is a step in that direction.

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Tables & Figures

Table 1: Frequency of Diagnosis Categories by Race

Diagnosis	Black	White
CPP	28.0% (14)[a]	22.5% (11)
OPP	18.0% (9)	16.3% (8)
PA	26.0% (13)	49.0% (24)
PT	28.0% (14)	12.2% (6)
Total	*100% (50)*	*100% (49)*

[a]Values in parentheses represent sample size (n), unless otherwise stated.

Table 2: Proportion of Patients with Evidence of Any Neurologic Damage or CNS Abnormality

Initial Diagnosis	White	Black
CPP	45.5% (5)	14.2% (2)
OPP	12.5% (1)	11.1% (1)
PA	12.8% (3)	23.1% (3)
PT	16.7% (1)	7.1% (1)
Total	*20.4% (10)*	*14.0% (7)*

Table 3: Summary Statistics of Patient BMI Z-Scores & Percentiles at Initial ECC Evaluation

Race	Diagnosis	Mean (SD)	Median	Min	Max	BMI>85th Percentile
Black						
	CPP (n=13)	1.47 (0.64)	1.40	0.48	2.47	76.9% (10)
	OPP (n=7)	2.01(0.70)	2.24	1.02	2.85	85.7% (6)
	PA (n=11)	1.26 (1.02)	1.49	-0.63	2.67	63.6% (7)
	PT (n=6)	0.94 (1.16)	0.68	-0.24	2.61	50.0% (3)
	Total (n=37)	*1.43 (0.90)*	*1.45*	*-0.63*	*2.85*	*70.3% (26)*
White						
	CPP (n=11)	0.96 (0.68)	0.90	0.03	2.35	36.4% (4)
	OPP (n=7)	1.49 (1.18)	1.78	-0.40	2.73	57.1% (4)
	PA (n=23)	0.74 (0.96)	0.85	-1.24	2.76	39.1% (9)
	PT (n=4)	1.05(1.28)	0.71	-0.07	2.84	25.0% (1)
	Total (n=45)	*0.94 (0.97)*	*0.85*	*-1.24*	*2.84*	*40.0% (18)*

SD=standard deviation; Min=Minimum; Max=Maximum

Table 4: Proportion of Patients in Low Income Medical Insurance Programs by Diagnosis

Race	CPP	OPP	PA	PT	Total
Black	50.0% (7)	66.7% (6)	46.2% (6)	92.9% (13)	*64.0% (32)*
White	36.4% (4)	25.0% (2)	16.7% (4)	16.7% (1)	*22.5% (11)*

Table 5: Summary Statistics of Patient Age (yrs) at Initial Evaluation for Precocious Puberty

Race	Diagnosis	Mean (SD)	Median	Min	Max
Black					
	CPP (n=14)	6.77 (2.10)	7.64	0.82	8.71
	OPP (n=9)	5.47 (2.87)	6.69	0.76	8.52
	PA (n=13)	7.11 (1.76)	7.35	4.90	9.95
	PT (n=14)	3.37 (2.51)	2.21	0.58	7.55
	Total (n=50)	*5.67 (2.71)*	*6.64*	*0.58*	*9.95*
White					
	CPP (n=11)	8.39 (1.09)	8.08	6.73	10.24
	OPP (n=8)	6.77 (2.35)	7.62	1.26	8.93
	PA (n=24)	7.30 (1.38)	7.35	2.88	9.94
	PT (n=6)	5.16 (2.80)	6.27	1.60	7.67
	Total (n=49)	*7.20 (1.91)*	*7.46*	*1.26*	*10.24*

Table 6: Summary Statistics of Estradiol Levels (pg/ml) by Diagnosis for Patients under 10 Years at the Time of Testing

Race	Diagnosis	Number Missing Estradiol	Mean (SD)	Median	Min	Max	>20 pg/ml[a]
Black							
	CPP (n=14)	0	24.67 (19.59)	20	<2[b]	63	42.9% (6)
	OPP (n=7)	2	18.40 (23.83)	14	<2[b]	69	28.6% (2)
	PA (n=5)	8	9.78 (4.47)	12	<7[b]	14	0% (0)
	PT (n=13)	1	13.34 (12.48)	14.14[c]	<2[b]	48	7.7% (1)
	Total (n=39)	*11*	*17.86 (17.46)*	*14*	*<2[b]*	*69*	*23.1% (9)*
White							
	CPP (n=9)	1	24.42 (9.60)	25	5.8	35	66.7% (6)
	OPP (n=8)	0	7.73 (5.23)	6.97	<3[b]	16.99	0% (0)
	PA (n=9)	14	15.57 (11.65)	13	*<2[b]*	40.74	33.3% (3)
	PT (n=6)	0	11.62 (11.73)	7.27	<3[b]	34.16	16.7% (1)
	Total (n=32)	*15*	*15.36 (11.35)*	*12.58*	*<2[b]*	*40.74*	*31.3% (10)*

[a]Dichotomized at the highest LOD in the sample.
[b]Undetected results were replaced with the value of the LOD divided by the square root of 2 to generate statistics.
[c]This median value corresponds to the estimate replacing the lab result recorded as <20 pg/ml with the LOD (20) divided by the square root of 2. Values surrounding this were 6 pg/ml and 16 pg/ml.

Figure 1: Distribution of Patient BMI Z-Scores at Initial ECC Evaluation

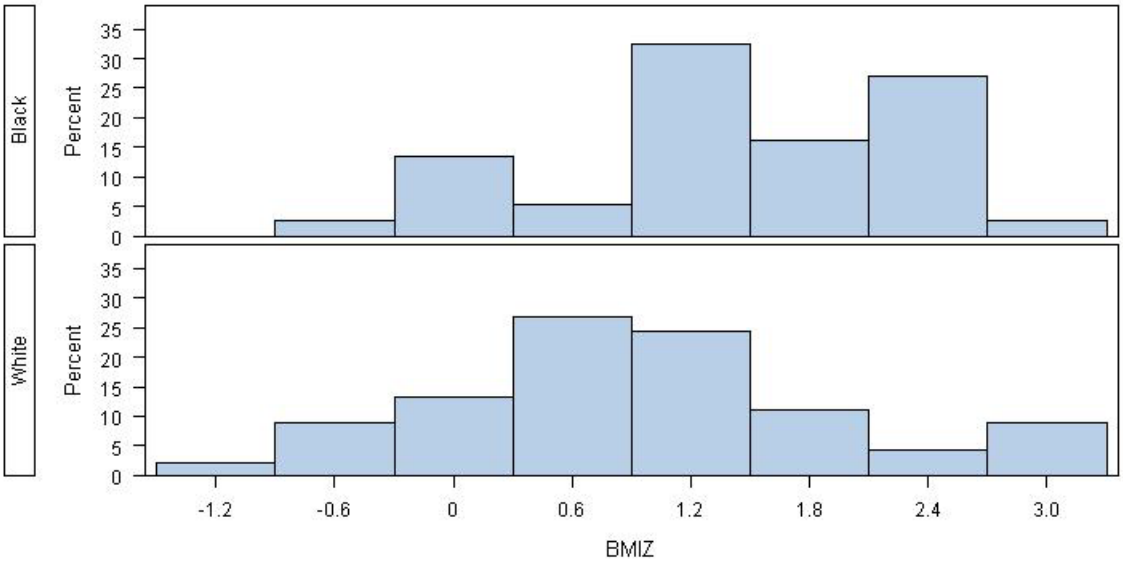
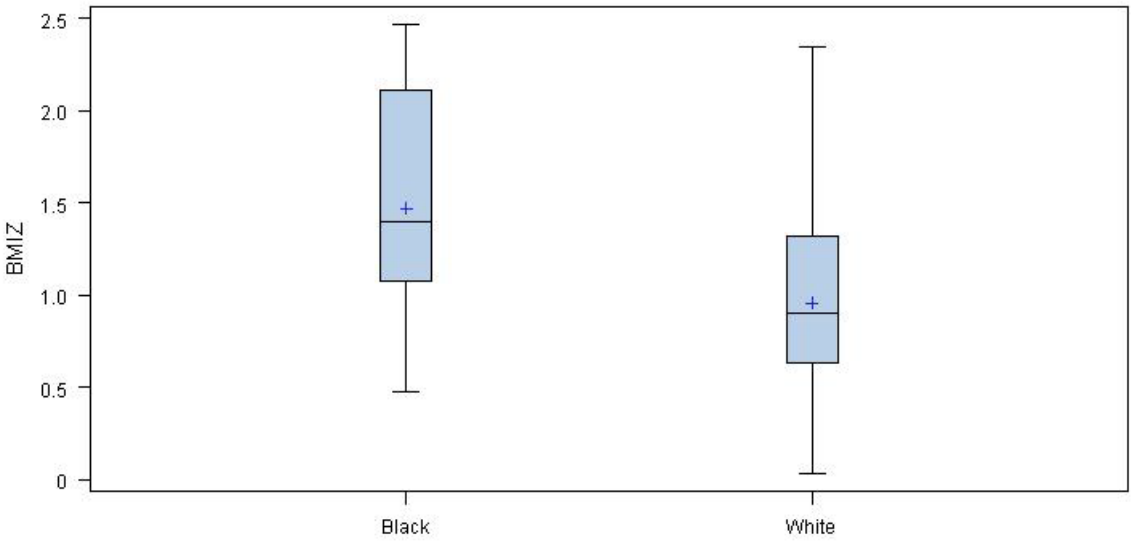
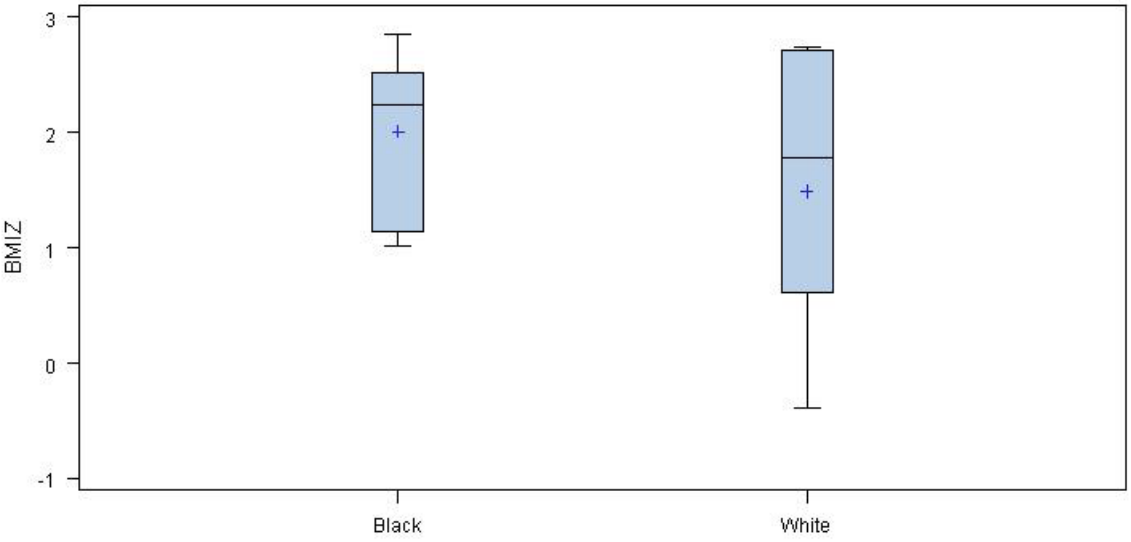


Figure 2: Z-Scores of Patient BMI at Initial ECC Evaluation by Diagnosis

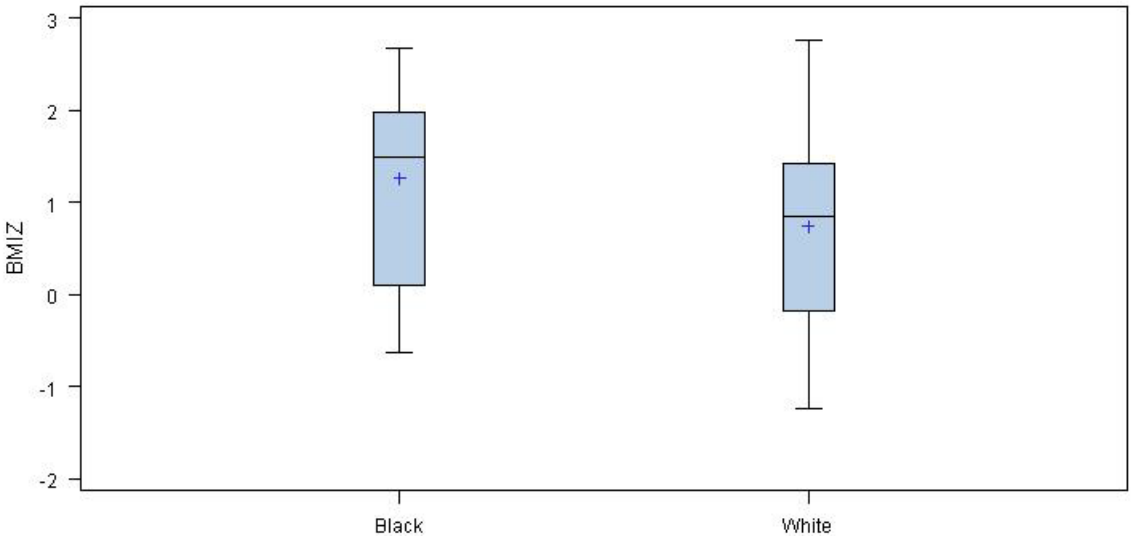
a) CPP



b) OPP



c) PA



d) PT

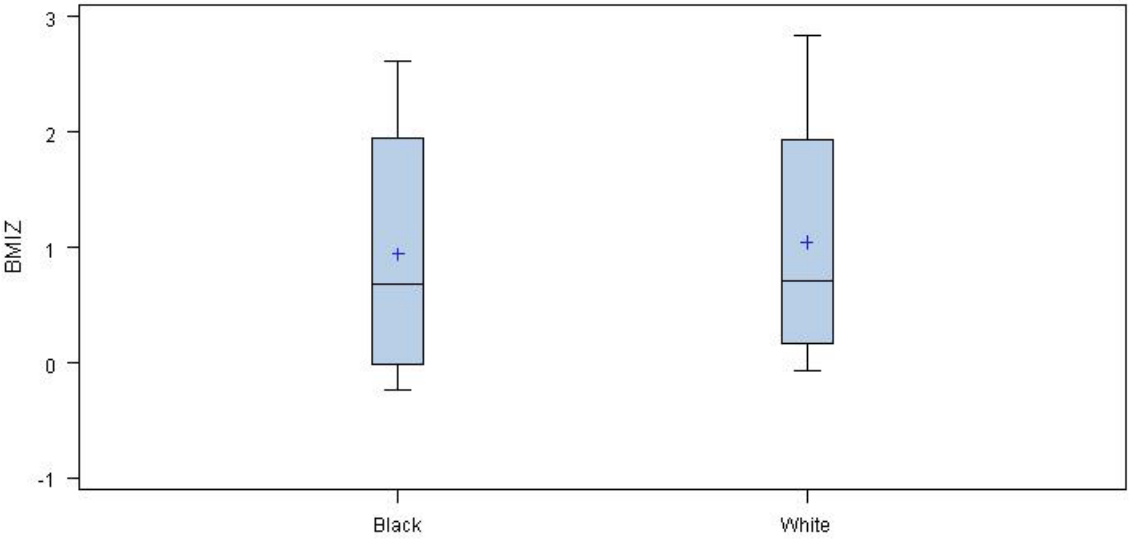


Figure 3: Distribution of Patient Ages at Initial Evaluation for Precocious Puberty

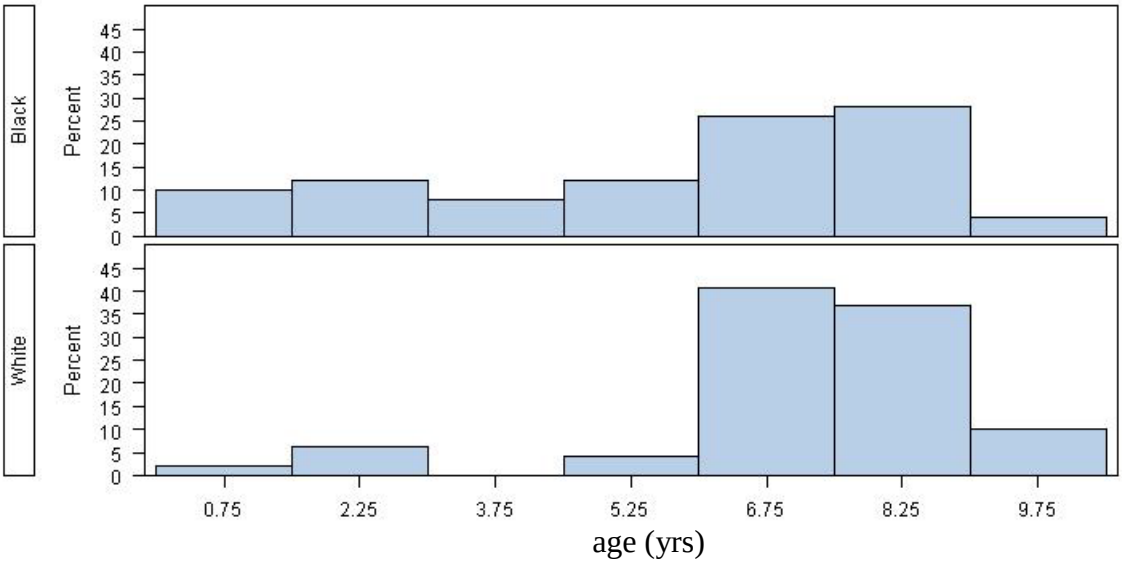
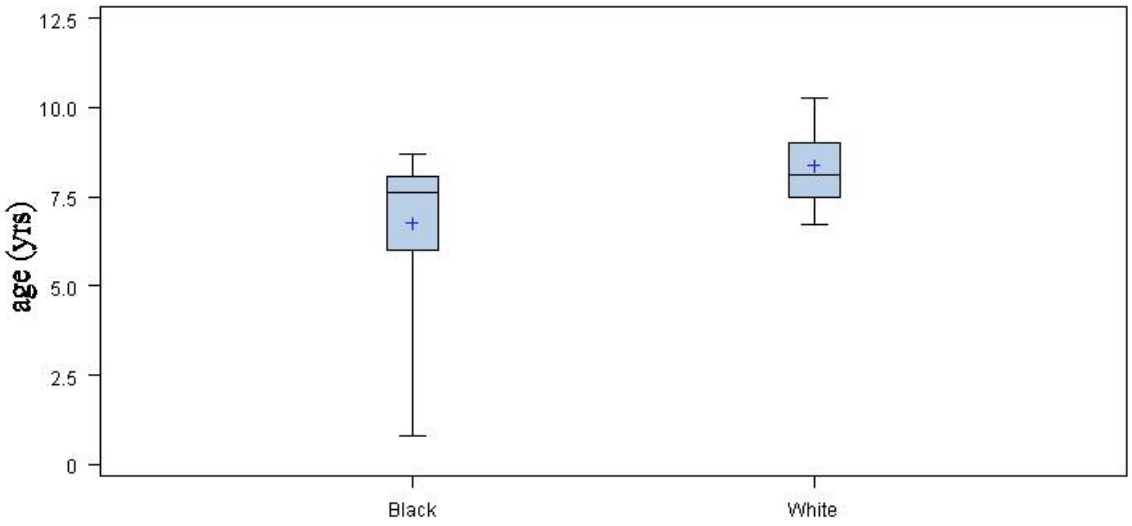
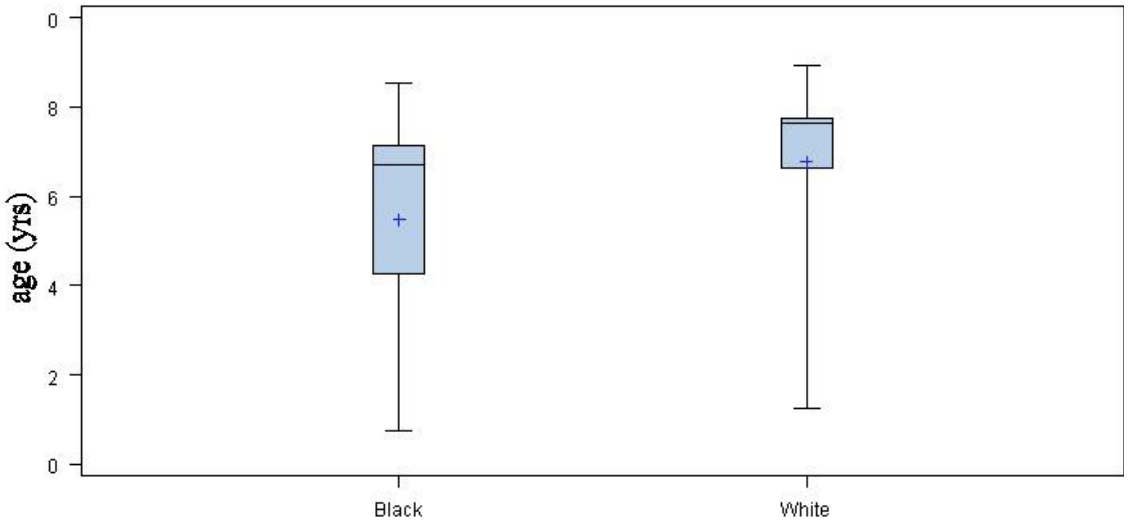


Figure 4: Age of Patients at Initial Evaluation for Precocious Puberty by Diagnosis

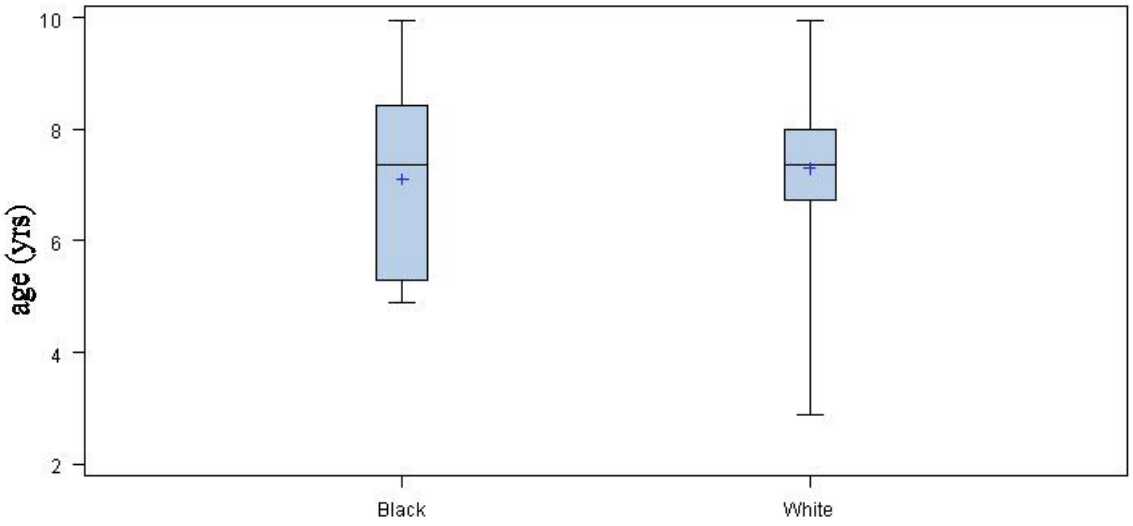
a) CPP



b) OPP



c) PA



d) PT

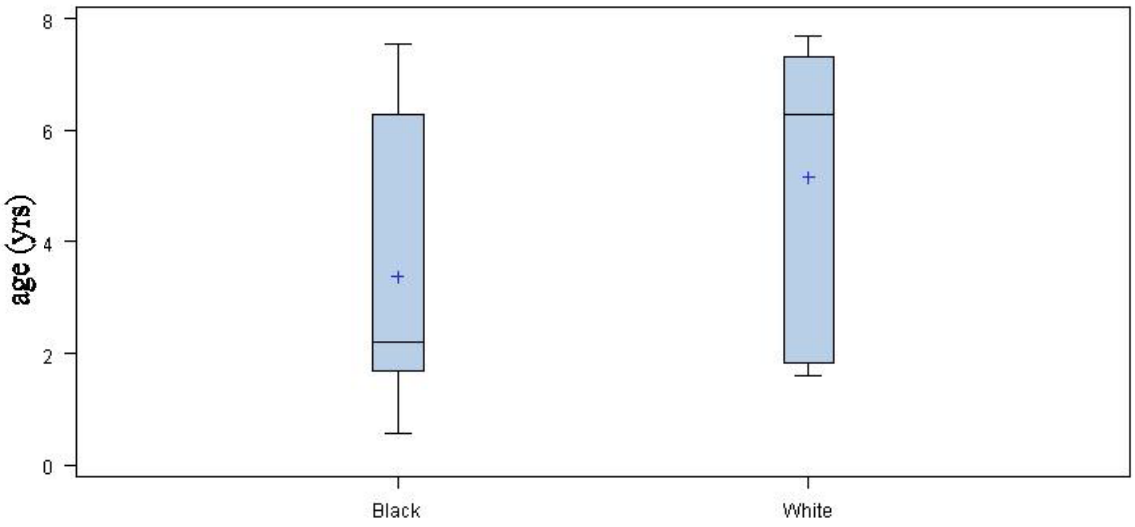


Figure 5: Distribution of Patients' First Estradiol Laboratory Result

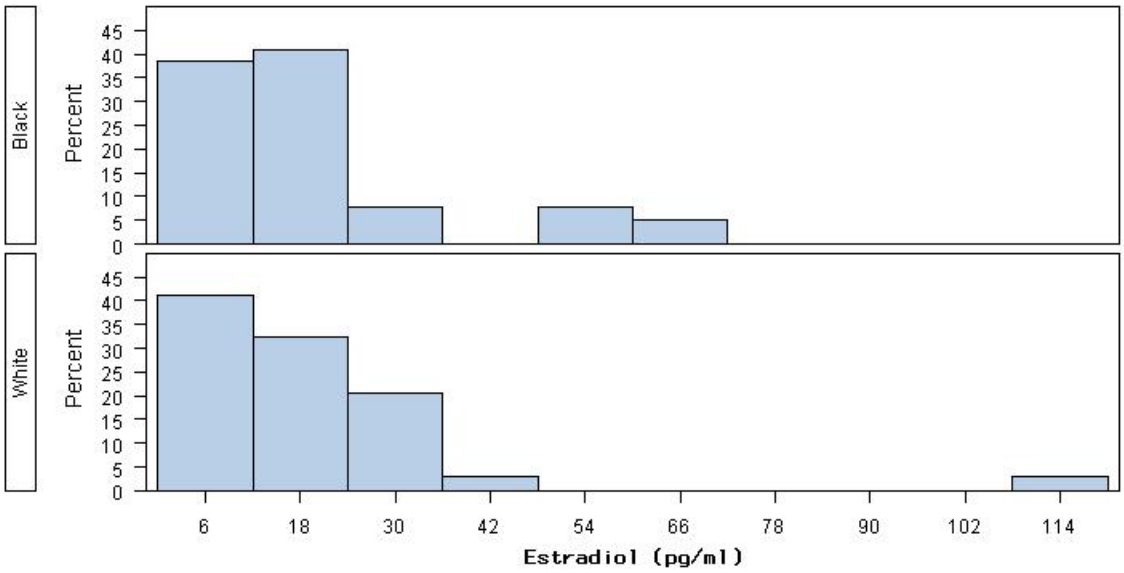
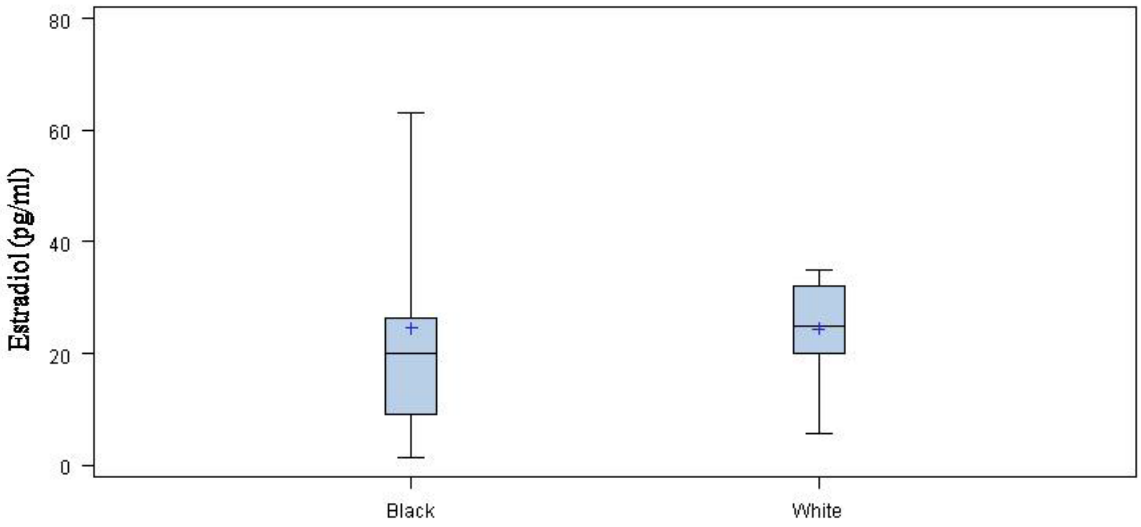
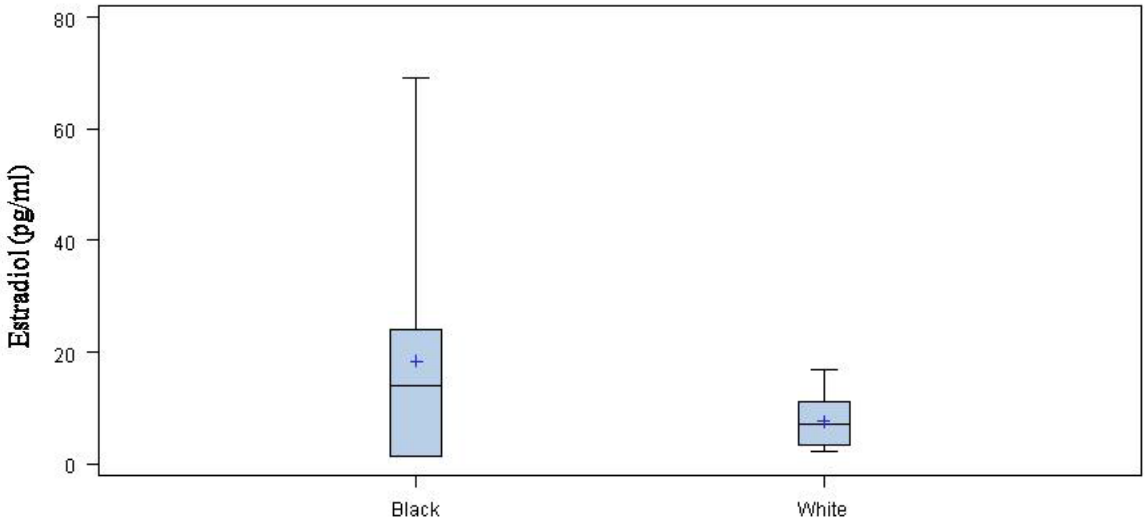


Figure 6: Estradiol Levels by Diagnosis for Patients under 10 Years at the Time of Testing

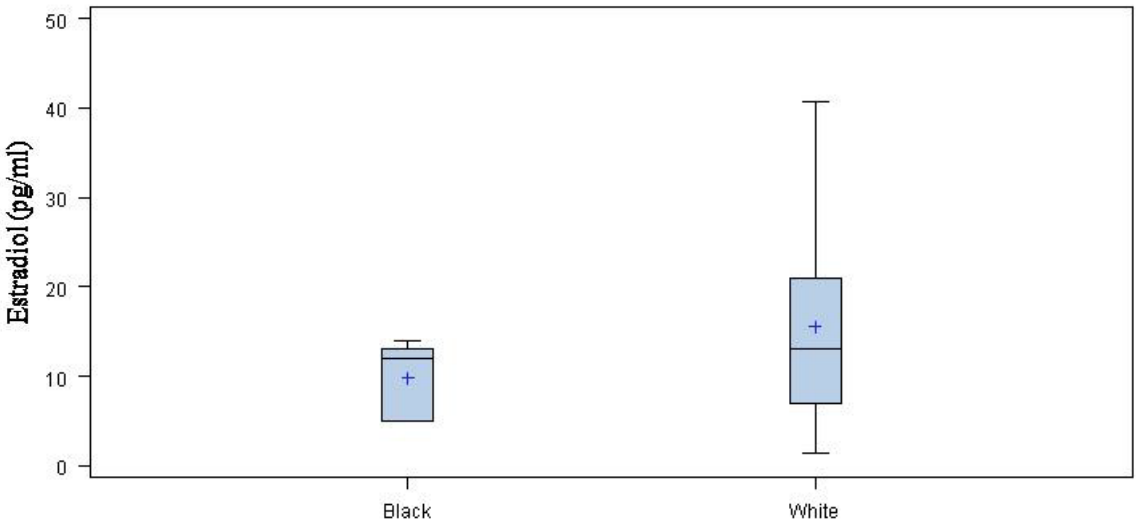
a) CPP



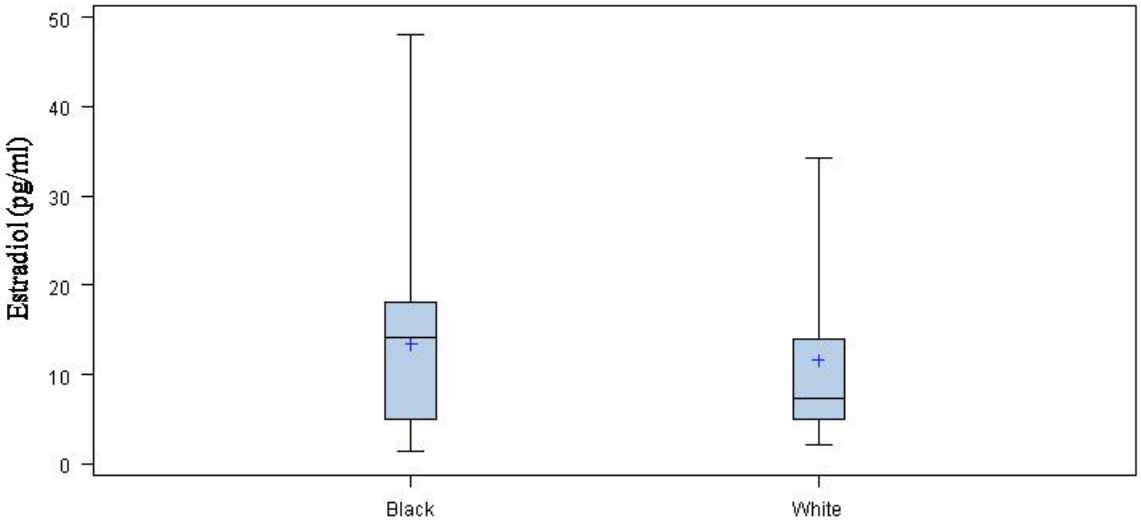
b) OPP



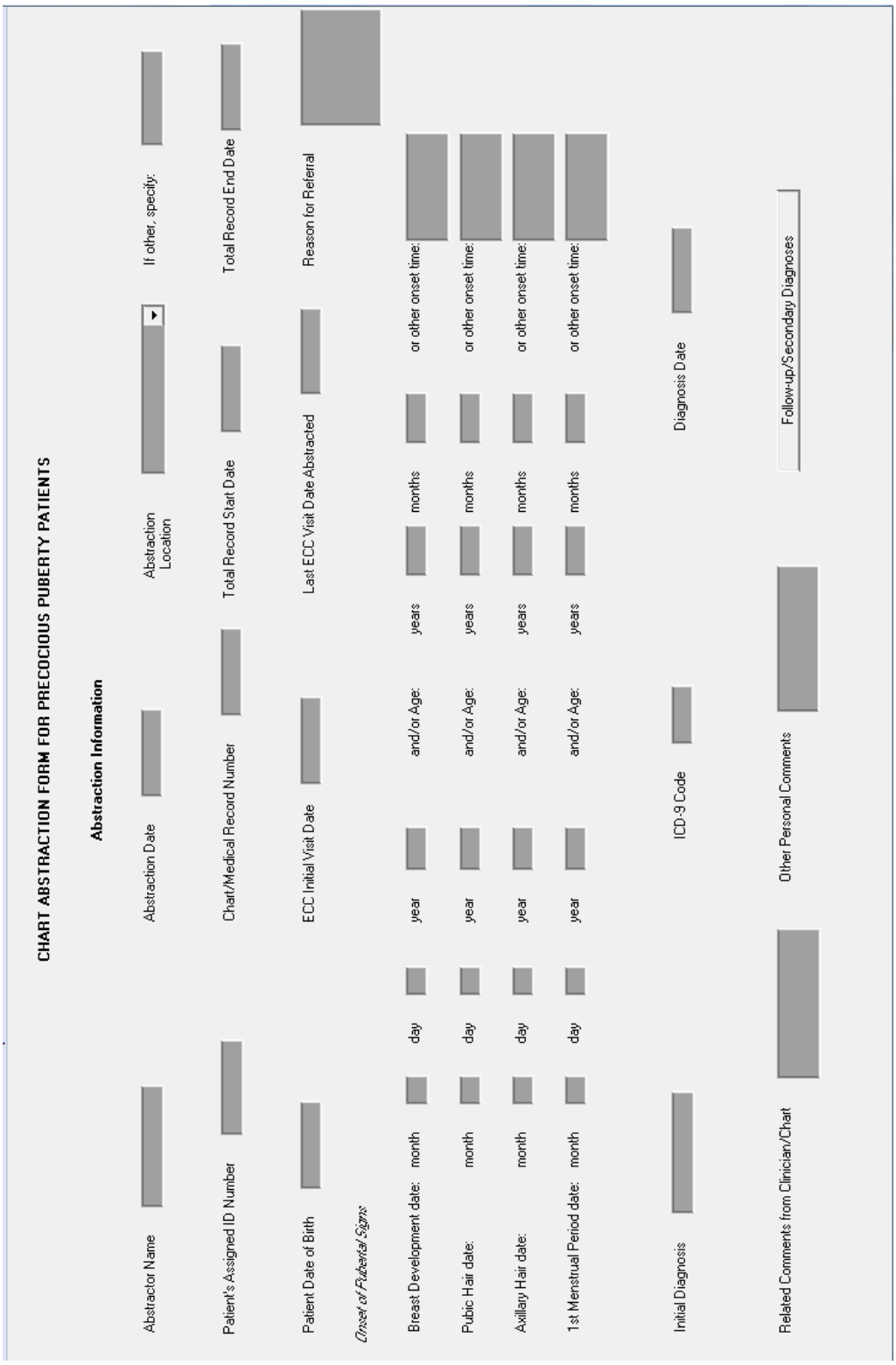
c) PA

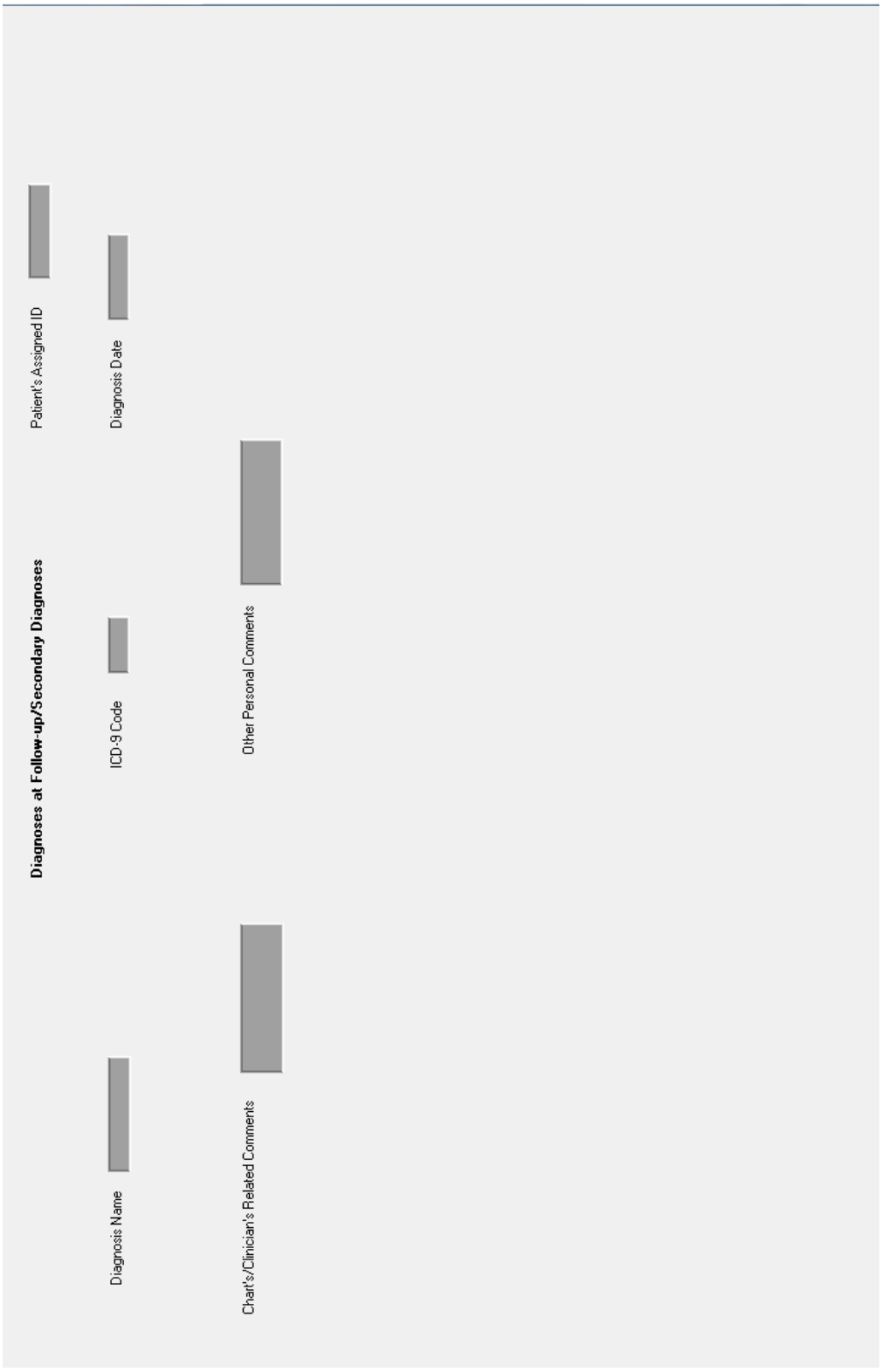

Estradiol (pg/ml)
50
40
30
20
10
0
Black
White

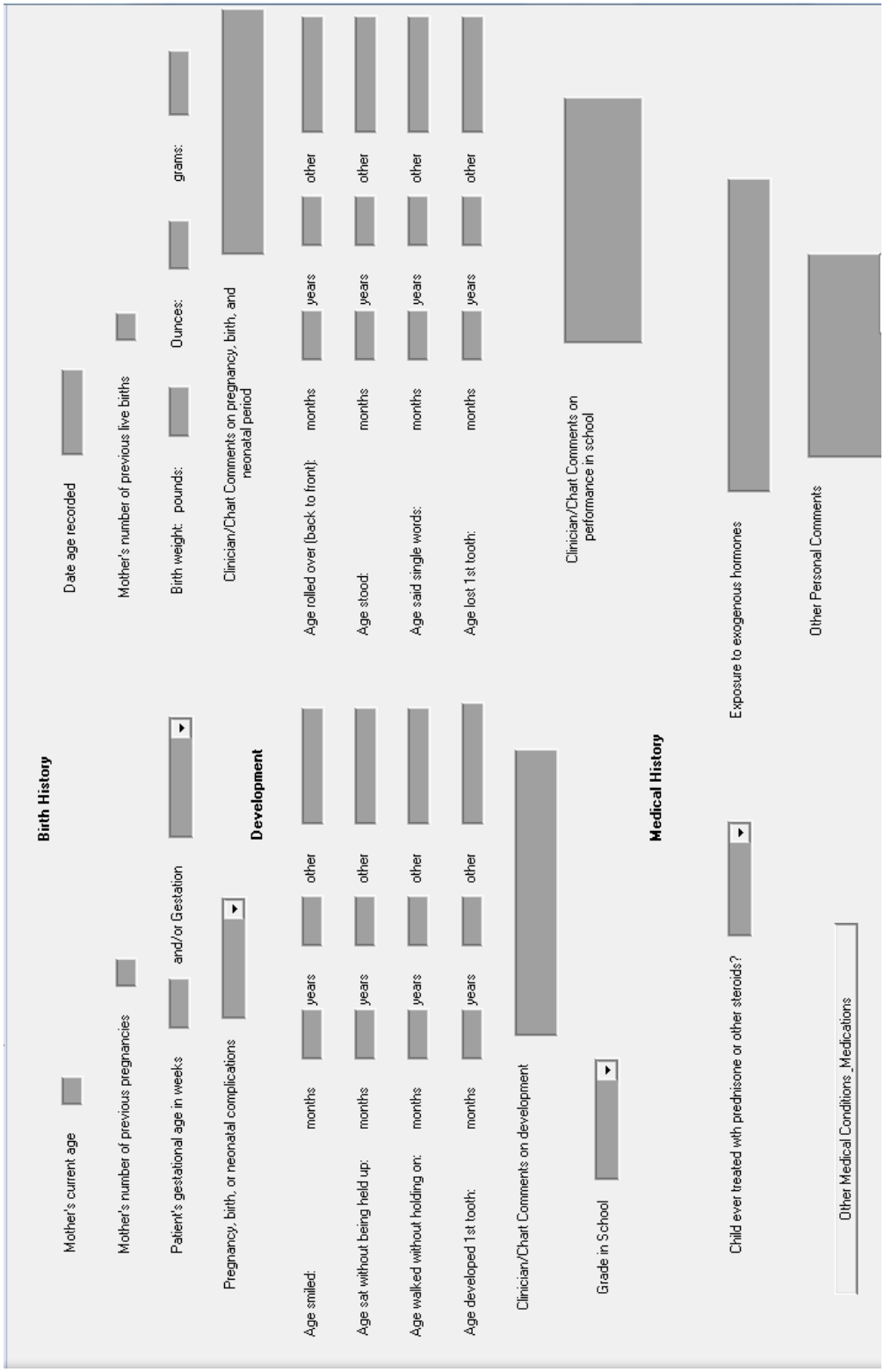
d) PT

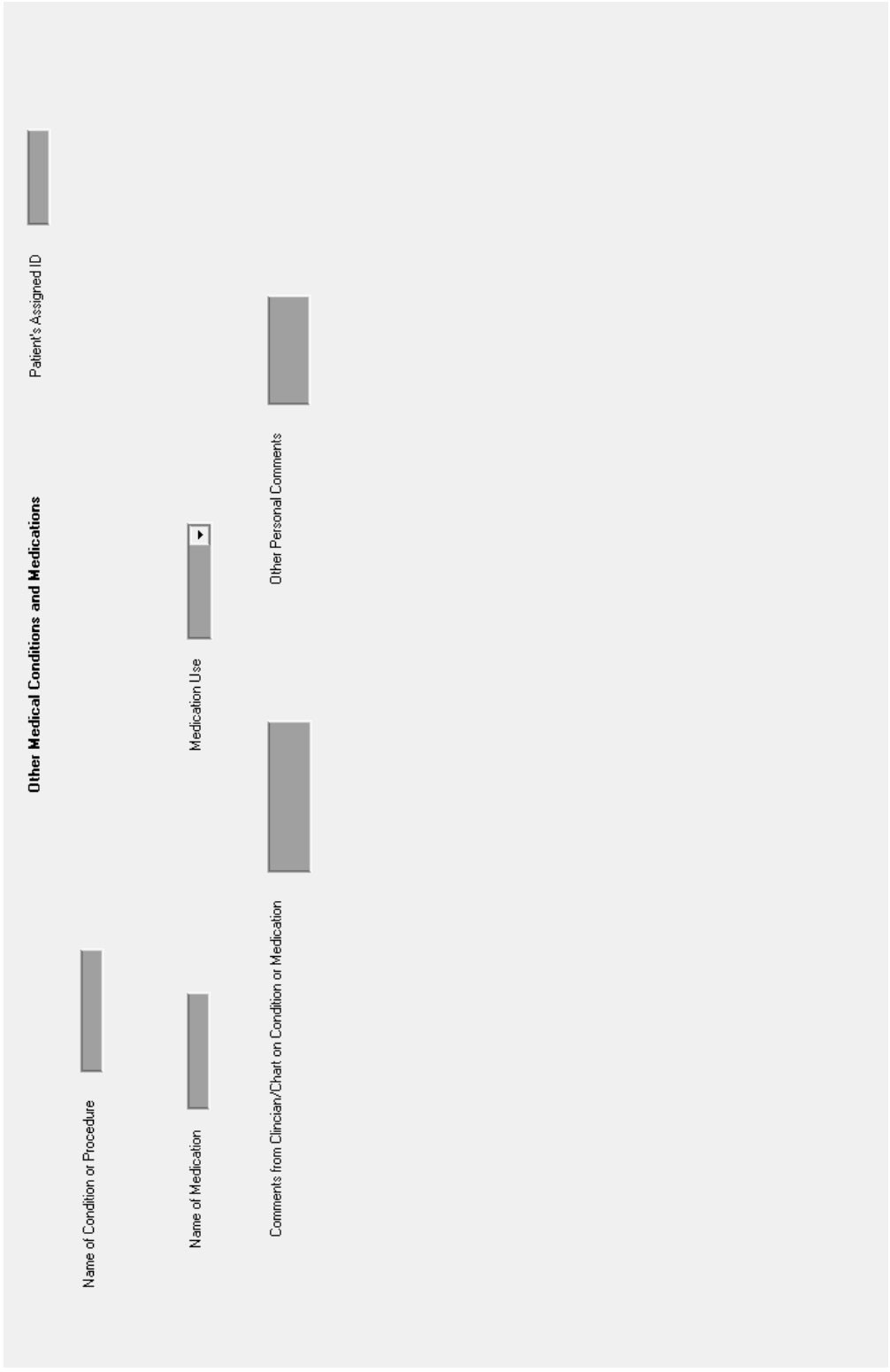

Estradiol (pg/ml)
50
40
30
20
10
0
Black
White

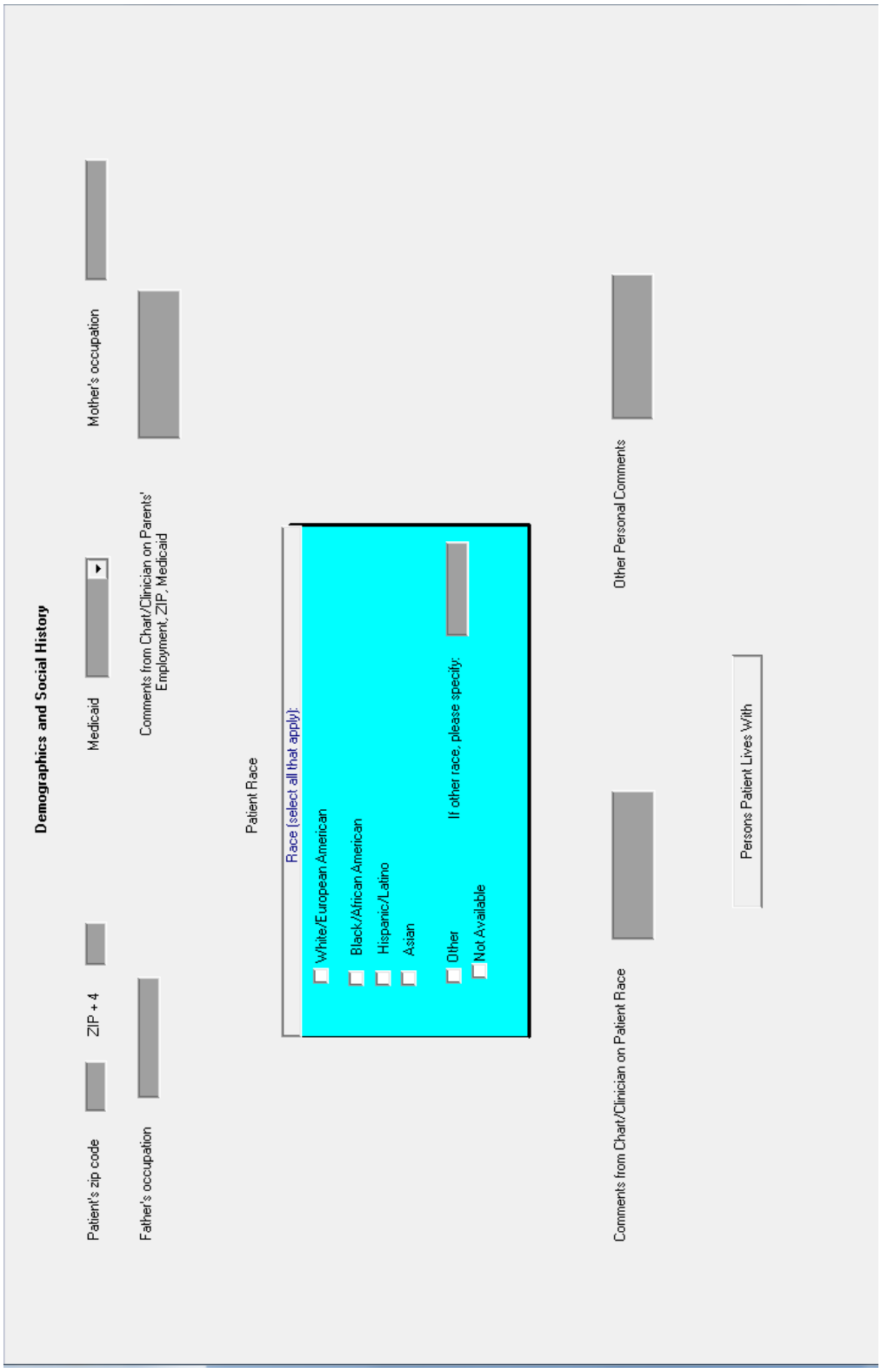
Appendix A: Abstraction Form

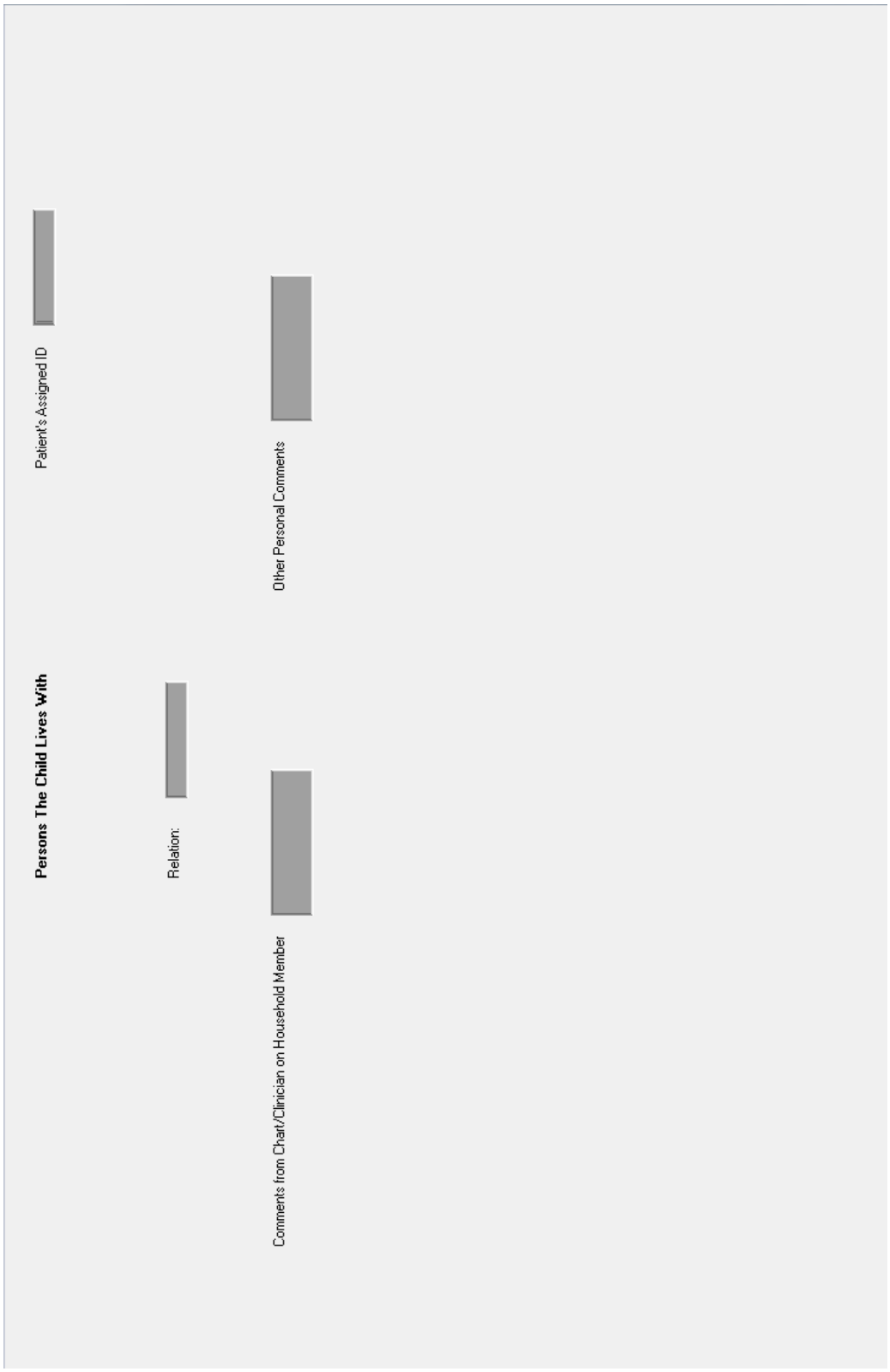


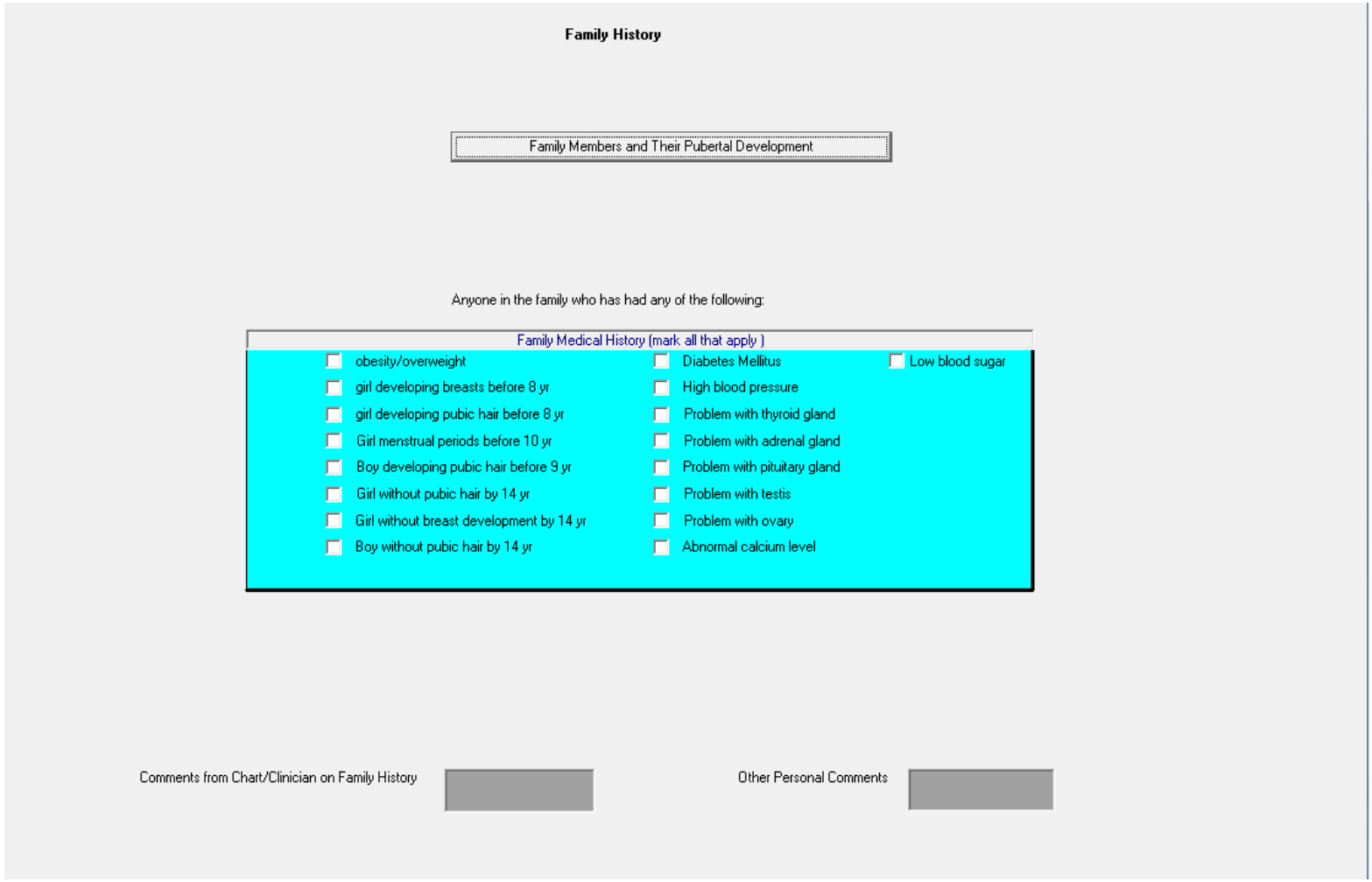

Diagnoses at Follow-up/Secondary Diagnoses
Patient's Assigned ID
Diagnosis Date
ICD-9 Code
Other Personal Comments
Diagnosis Name
Chart's/Clinician's Related Comments

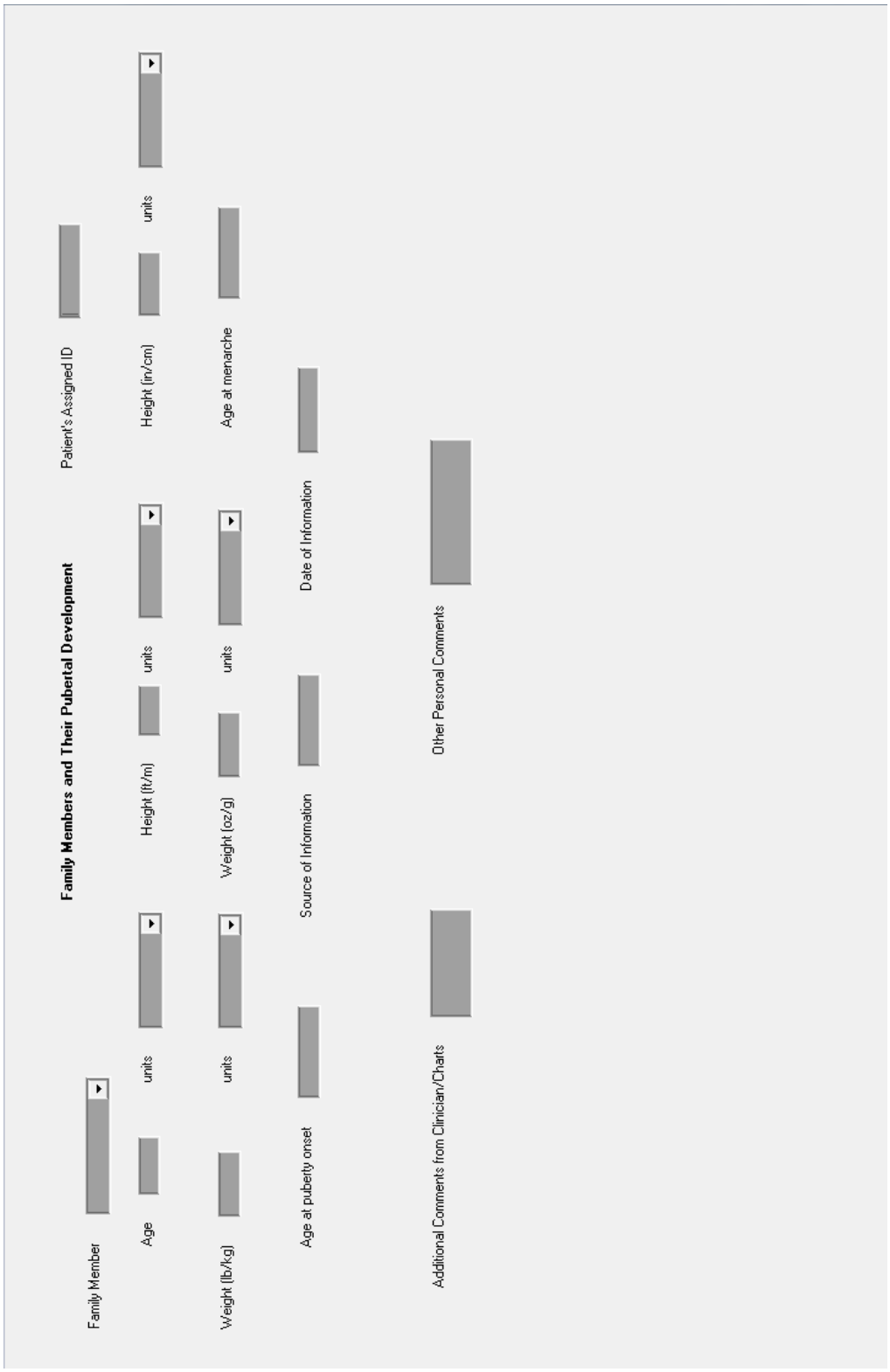

Birth History
Mother's current age
Date age recorded
Mother's number of previous pregnancies
Mother's number of previous live births
Patient's gestational age in weeks and/or Gestation
Birth weight: pounds: Ounces: grams:
Pregnancy, birth, or neonatal complications
Clinician/Chart Comments on pregnancy, birth, and neonatal period
Development
Age smiled: months years other
Age rolled over (back to front): months years other
Age sat without being held up: months years other
Age stood: months years other
Age walked without holding on: months years other
Age said single words: months years other
Age developed 1st tooth: months years other
Age lost 1st tooth: months years other
Clinician/Chart Comments on development
Clinician/Chart Comments on performance in school
Grade in School
Medical History
Child ever treated with prednisone or other steroids?
Exposure to exogenous hormones
Other Personal Comments
Other Medical Conditions_Medications

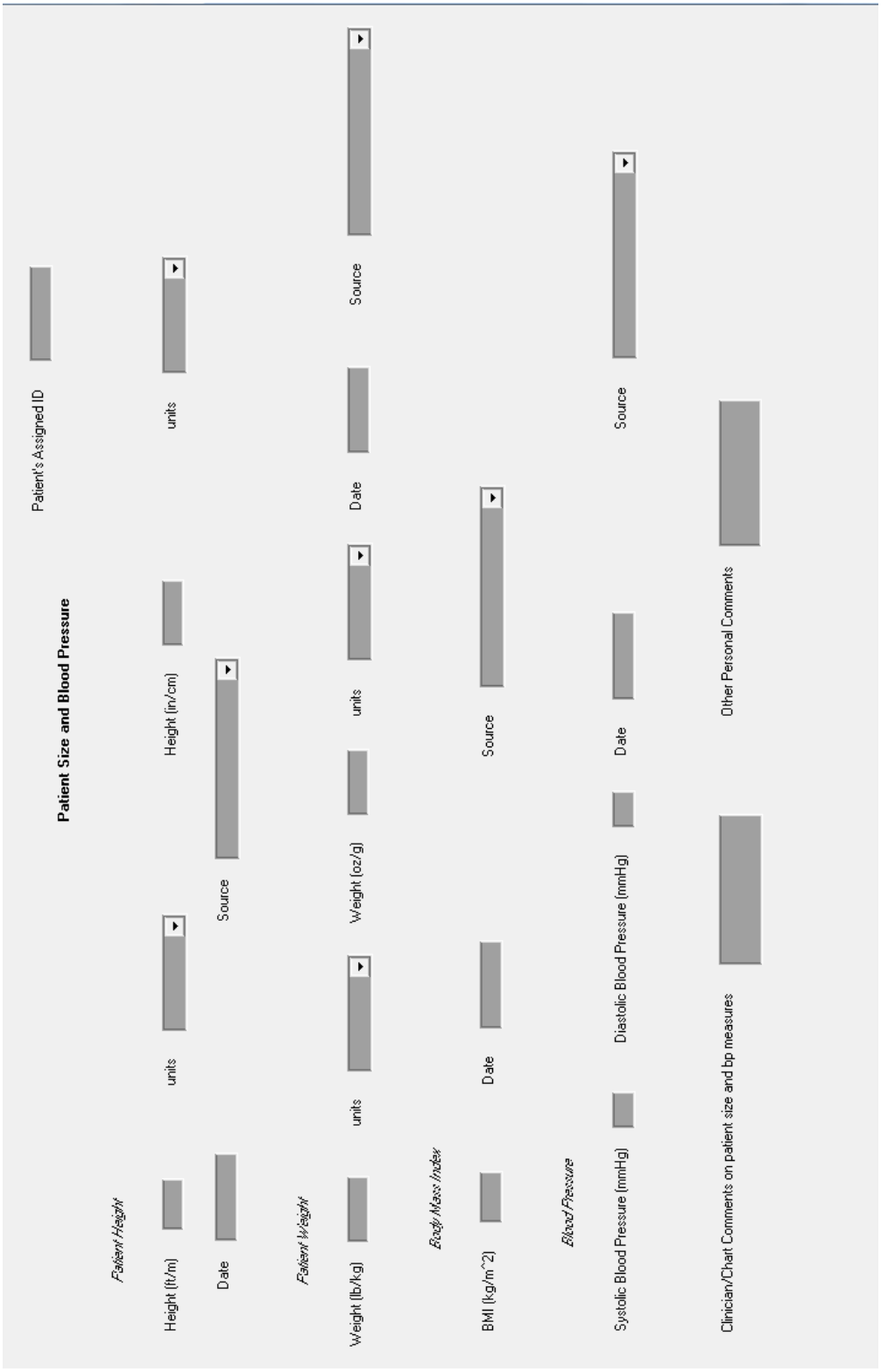

Other Medical Conditions and Medications
Patient's Assigned ID
Name of Condition or Procedure
Name of Medication
Medication Use
Comments from Clinician/Chart on Condition or Medication
Other Personal Comments


Demographics and Social History
Patient's zip code
ZIP + 4
Medicaid
Mother's occupation
Father's occupation
Comments from Chart/Clinician on Parents' Employment, ZIP, Medicaid
Patient Race
Race (select all that apply):
White/European American
Black/African American
Hispanic/Latino
Asian
Other
If other race, please specify:
Not Available
Other Personal Comments
Comments from Chart/Clinician on Patient Race
Persons Patient Lives With


Patient's Assigned ID
Other Personal Comments
Persons The Child Lives With
Relation:
Comments from Chart/Clinician on Household Member


Family History
Family Members and Their Pubertal Development
Anyone in the family who has had any of the following:
Family Medical History (mark all that apply)
obesity/overweight
girl developing breasts before 8 yr
girl developing pubic hair before 8 yr
Girl menstrual periods before 10 yr
Boy developing pubic hair before 9 yr
Girl without pubic hair by 14 yr
Girl without breast development by 14 yr
Boy without pubic hair by 14 yr
Diabetes Mellitus
High blood pressure
Problem with thyroid gland
Problem with adrenal gland
Problem with pituitary gland
Problem with testis
Problem with ovary
Abnormal calcium level
Low blood sugar
Comments from Chart/Clinician on Family History
Other Personal Comments


Family Members and Their Pubertal Development
Patient's Assigned ID
Family Member
Age
units
Height (ft/m)
units
Height (in/cm)
units
Weight (lb/kg)
units
Weight (oz/g)
units
Age at menarche
Age at puberty onset
Source of Information
Date of Information
Other Personal Comments
Additional Comments from Clinician/Charts


Patient Size and Blood Pressure
Patient's Assigned ID
Patient Height
Height (ft/in)
units
Height (in/cm)
units
Date
Source
Patient Weight
Weight (lb/kg)
units
Weight (oz/g)
units
Date
Source
Body Mass Index
BMI (kg/m^2)
Date
Source
Blood Pressure
Systolic Blood Pressure (mmHg)
Diastolic Blood Pressure (mmHg)
Date
Source
Clinician/Chart Comments on patient size and bp measures
Other Personal Comments

Assessment of Pubertal Status

Patient's Assigned ID

Tanner Stages for Breast Development

Breast Stage (or if not single number): Date Source

Tanner Stages for Pubic Hair Development

Pubic Hair Stage (or if not single number): Date Source

Patient Bone Age

Bone age years Bone age months Date Location taken

Read by Location read

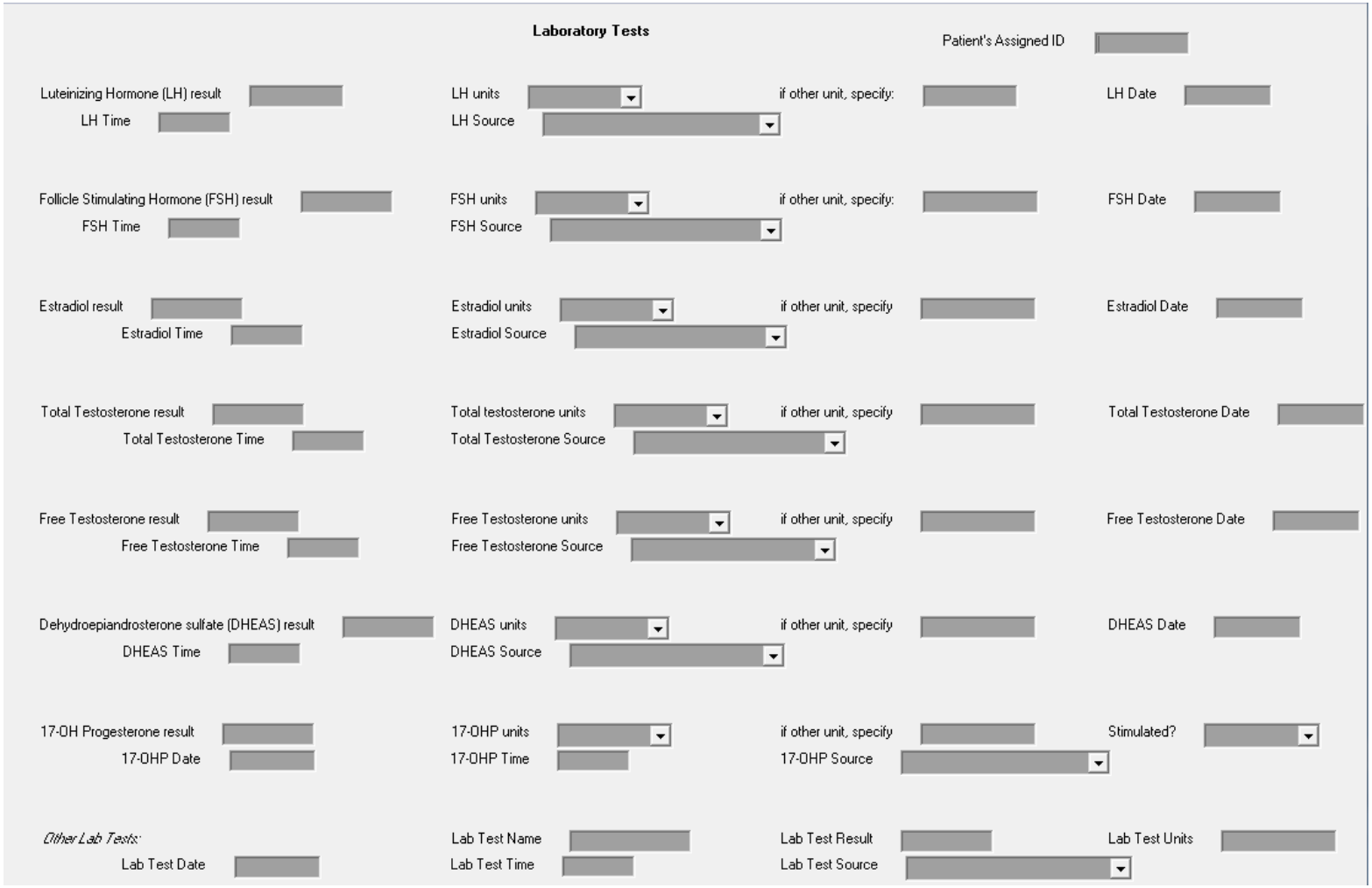
(if bone age read multiple times and interpretation differs, enter 2nd interpretation below):

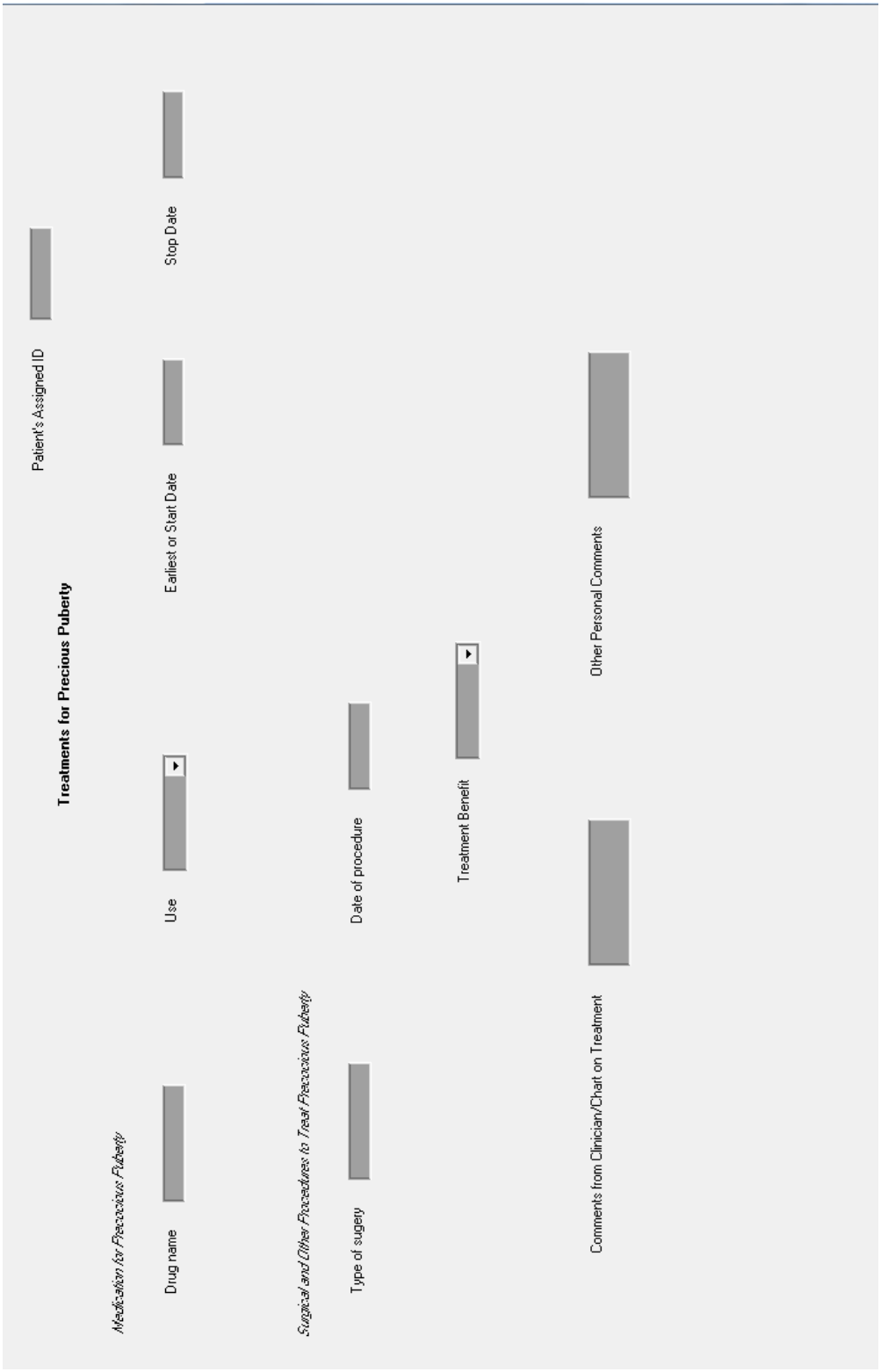
Bone age years Bone age months Date Location Taken

Read by Location read

Clinician/Chart Comments on pubertal features

Other Personal Comments


Laboratory Tests
Patient's Assigned ID
Luteinizing Hormone (LH) result
LH units
if other unit, specify:
LH Date
LH Time
LH Source
Follicle Stimulating Hormone (FSH) result
FSH units
if other unit, specify:
FSH Date
FSH Time
FSH Source
Estradiol result
Estradiol units
if other unit, specify
Estradiol Date
Estradiol Time
Estradiol Source
Total Testosterone result
Total testosterone units
if other unit, specify
Total Testosterone Date
Total Testosterone Time
Total Testosterone Source
Free Testosterone result
Free Testosterone units
if other unit, specify
Free Testosterone Date
Free Testosterone Time
Free Testosterone Source
Dehydroepiandrosterone sulfate (DHEAS) result
DHEAS units
if other unit, specify
DHEAS Date
DHEAS Time
DHEAS Source
17-OH Progesterone result
17-OHP units
if other unit, specify
Stimulated?
17-OHP Date
17-OHP Time
17-OHP Source
Other Lab Tests:
Lab Test Name
Lab Test Result
Lab Test Units
Lab Test Date
Lab Test Time
Lab Test Source


Patient's Assigned ID
Treatments for Precious Puberty
Medication for Precocious Puberty
Drug name
Use
Earliest or Start Date
Stop Date
Surgical and Other Procedures to Treat Precocious Puberty
Type of sugery
Date of procedure
Treatment Benefit
Other Personal Comments
Comments from Clinician/Chart on Treatment